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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/29, 15/11, 15/82, 5/10, 1/21, C12Q 1/68, C07K 14/415, 16/16, A01H 5/00, G01N 33/50

A2

(11) International Publication Number: WO 98/00545

(43) International Publication Date: 8 January 1998 (08.01.98)

(21) International Application Number: PCT/GB97/01766

(22) International Filing Date: 30 June 1997 (30.06.97)

(30) Priority Data:

9613617.1	28 June 1996 (28.06.96)	GB
9706118.8	25 March 1997 (25.03.97)	GB

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

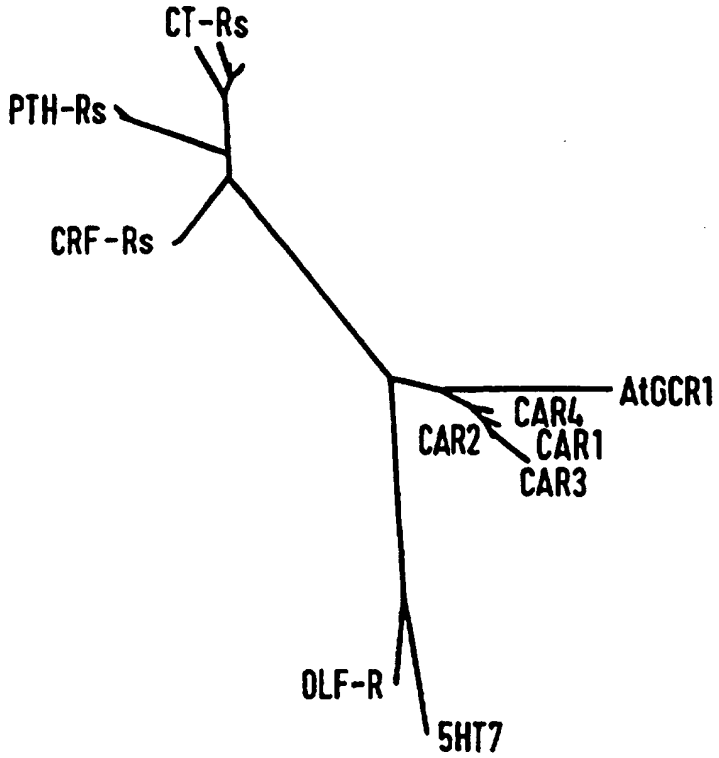
Published

Without international search report and to be republished upon receipt of that report.

(54) Title: G PROTEIN SIGNAL TRANSDUCTION IN PLANTS

(57) Abstract

A method of altering G protein signal transduction pathways in plants and thereby influencing plant growth, development and responses to endogenous signalling molecules, environmental signals, pests and pathogens is disclosed herein. A G protein-coupled receptor in *Arabidopsis thaliana*, *Brassica napus* and *Brassica oleracea* have been discovered, isolated and cloned. Specifically, the cDNA sequence encoding the full length receptor *Arabidopsis thaliana* has been isolated and purified, as well as the amino acid sequence. The isolated G protein-coupled receptor of the present invention is believed to influence the sensitivity of plants to cytokinins.



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G PROTEIN SIGNAL TRANSDUCTION IN PLANTS

This invention relates to the application of recombinant DNA technology to plants, specifically, to the discovery, isolation and cloning of a G protein-coupled receptor homologue for mediating both G protein signal transduction pathways and G protein-independent signal transduction pathways in plants.

G protein-coupled receptors (GPCRs) constitute a large and functionally diverse superfamily of integral membrane proteins involved in the transduction of signals across cell membranes. The G-protein signalling pathway is also one of the most highly conserved mechanisms for transducing extracellular signals. The GPCRs are an essential element of this pathway, although to date, they have not been isolated from plants.

The action of a wide variety of extracellular signalling molecules is mediated specifically by GPCRs. These signals include olfactrants, biogenic amines, vertebrate and invertebrate visual pigments, neuropeptides, a range of autocrine, paracrine and endocrine factors, insect pheromones, fungal mating factors and chemotactic substances. Despite the diversity of agonists that stimulate GPCRs, these receptors share considerable structural homology, reflecting their common mechanism of action. The DNA and deduced protein sequence of more than 700 GPCRs are known with all sharing a common feature of 7 stretches of hydrophobic amino acids each capable of forming a transmembrane α-helix. Thus, GPCRs are typically thought to traverse the membrane 7 times, forming a helical cluster with associated extracellular and intracellular loops. This hypothesis is supported by a number of molecular modelling studies (Strader *et al.*, *Ann. Review of Biochem.* 63:101-132 (1994)).

GPCRs may be classified into families on the basis of primary sequence homology in the transmembrane domains. In general, these homologies are greatest for species homologues (85-90% identity), and sub-types of the same receptor (60-80% identity). Members of the same family can show as low as 35-40% identity and unrelated GPCRs may be only 20-25% identical in the transmembrane domains. In addition to the homologies within the transmembrane regions there are a number of highly conserved residues within the loop regions, some of which are conserved between families. Currently, six families of GPCRs are recognised and the receptors in each of these families are thought to share a common ancestor.

GPCRs have been identified in vertebrates, invertebrates, arthropods, insects, nematodes, fungi, yeast, and viruses, but never in plants. However, a rhodopsin-regulated calcium current has been demonstrated in *C. reinhardtii* (a green algae), suggesting that evolution of GPCR-mediated transmembrane signalling may have predated the separation of plants from animals (Harz, H. and P. Hegeman, *Nature* 35:489-91 (1991)).

In signal transduction, ligand binding occurs through a variety of mechanisms, depending on the class of ligand. The cavity formed by the cluster of transmembrane helices appears to be involved in binding ligands such as adrenaline, serotonin and acetylcholine. Some of the peptide receptors bind ligands through the exofacial regions of the extracellular loops. Other GPCRs appear to use long N-terminal extracellular domains in a variety of ways to both bind and orientate the ligand with respect to the extracellular face and transmembrane regions of the receptor.

It is believed that GPCRs signal through heterotrimeric GTP-binding proteins (G proteins) which comprise α, β and γ subunits. It appears that the three cytoplasmic loops and the cytoplasmic ends of the transmembrane helices undergo a conformational change when a ligand is bound by the receptor and this allows G proteins to interact particularly with the third and second intracellular loops, and the C-terminus. The G protein then exchanges bound GDP for GTP and in this activated form, dissociates into the α-subunit and $\beta\gamma$-complex, both of which can interact with effectors such as ion channels or second messenger-generating enzymes and thus impact on signal transduction cascades. It is also thought the GPCRs additionally have the capacity to mediate signalling events independentaly of G proteins. For example, GPCR-mediated, G protein-independent calcium influx (C.A. Parent & P. N. Devreotes, *Ann. Rev. Biochem*, **65**:411-440 (1996).

There are far fewer classes of G proteins than GPCRs, probably no more than a dozen in vertebrates. This suggests that different classes of GPCRs signal through a single class of G protein. Thus, receptors determine the specificity of the response, and the G proteins are "multifunctional" signalling molecules. Therefore, in order to specifically manipulate G protein signal transduction, attention must be focused on the G protein-coupled receptors and their ligands.

Prior to this invention, only circumstantial evidence existed suggesting that G proteins might be present in plants, however, only minimal evidence existed to support the presence of plant G protein-coupled receptors. H. Ma, *Plant Mol. Biol.*, **26**:1611-1636 (1994). For instance,

in studies related to red and blue light signal transduction in plants, cholera and pertussis toxins were shown to uncouple phytochrome-dependent gene expression in dark-adapted soybean cells (Muschietti *et al.*, *J. Biochem.*, **291**:383-388 (1993)). In other studies, the use of G protein agonists and antagonists in association with whole-cell patch clamped *Vicia faba* guard cells and mesophyll protoplasts, indicated that inward and outward rectifying K^+ channels were possibly regulated by G proteins(S.M.Assman, *TIPS* 1:73-74 (1996)). The expression pattern of the Gα subunit in *Arabidopsis* also indicated possible involvement in the regulation of cell division and differentiation (Weiss *et al*, *The Plant Cell*, 5: 1513-1528 (1993)).

A functional association between red-light photoreceptors and G proteins was also inferred from studies with *Medicago sativa*. Protoplasts isolated from etiolated wheat leaves swell in response to a variety of stimuli including plant hormones and red light. This calcium-dependent response was inhibited by $GDP_\beta S$, while $GTP_\gamma S$ induced swelling to the same extent as red light. Moreover, plasma membranes and apical buds of dark-grown *P. sativum* have also been shown to contain GTPase activity stimulated by low-influence blue light. Lastly, a 40 kDa polypeptide which cross-reacted with G_α-specific antisera and was ADP-ribosylated by cholera and pertussis toxins, suggested that this peptide was a G protein (H. Ma, *Plant Mol. Biol.*, **26**:1611-1636 (1994)). However, a definitive isolation and characterisation of a GPCR in plants was never made prior to this invention.

We believe that GPCRs in plants will function similarly to their vertebrate and invertebrate counterparts, for

instance, in responding to fungal elicitors, light, responding to plant hormones, as well as regulating cell division and differentiation. The use of such plant GPCRs would be of great benefit to plant breeders. For example, by controlling and/or manipulating plant G protein signal transduction pathways through alteration of GPCR expression, sensitivity, activity or sepcificity might allow plant growers to manipulate plant responses to both light quality and quantity and thus, alter the transition from vegetative to reproductive growth--hence delaying or advancing flowering. It may also be possible to influence the sensitivity to UV/blue light which may have important long term implications in relation to ozone layer depletion in the atmosphere.

Additionally, by isolating G protein-coupled receptors in plants, the agricultural and horticultural industries would have the ability to manipulate responses to plant hormones by altering G protein signal transduction and likely affect events such as: seed dormancy, germination, elongation growth, responses to stress, flower initiation and development, and fruit ripening and development. Further, by, for example, altering GPCR expression, sensitivity, activity or specificity, it could be possible to manipulate ion/nutrient transport, disease resistance and response to pathogens as well as cellular division and differentiation. Other signals in plants possibly involving G proteins are hormones such as auxin, gibberellin and abscisic acid, as well as fungal elicitors.

The present invention is based on the first ever isolation of a G protein-coupled receptor homologue in plants. Specifically, this invention relates to the

isolation of a novel and useful gene to a plant G protein-coupled receptor homologue from the family *Cruciferae* and relates to methods of isolation, genetic constructs and transgenic plants containing this gene.

According to the invention, there are provided nucleic acid and amino acid sequences encoding a G protein-coupled receptor homologue. This novel GPCR homologue gene, referred herein as *AtGCR1*, was isolated from the plant *Arabidopsis thaliana*. The DNA and amino acid sequences of Fig. 1 and Fig 2 and sequences substantially homologous to the sequences of Fig 1 and Fig. 2 (i.e. also encoding a plant G protein-coupled receptor homologue) are claimed by way of this invention. The nucleotide and amino acid sequences forming part of this invention, may in principle, be isolated from any fungi or plant.

The plant G protein-coupled receptor homologue of Fig 1 and Fig. 2 influences sensitivity to cytokinins in plants, specifically in *Arabidopsis*. Cytokinins are purine derivatives and are a profoundly important class of plant hormones and are known to influence cell division, differentiation, senescence, responses to light, flowering and apical dominance (See: *Plant Growth Substances 1988*, Springer-Verlag Publishers, R.D. Carlson and A.J. Crovetti, p.p. 604-610 and Gan, S. & Amasino, R.M. *Bioassays* **18**: 557-565 (1996)). Prior to this invention, there was little, if any, evidence in the literature that cytokinin responses were actually mediated by G proteins or G protein-coupled receptor homologues. The discovery that the *AtGCR1* gene of this invention affects the sensitivity of plants to cytokinins generally, and to benzyladenine specifically, is fully

described in the embodiments section of this specification and also forms part of this invention.

A preferred aspect of this invention is, therefore, a DNA sequence or a fragment of a DNA sequence encoding a GPCR homologue isolated from fungi or plants, preferably from monocotyledonous or dicotyledonous plants, more preferably from dicotyledonous plants, or a sequence having substantial homology thereto and wherein said DNA sequence is capable in plants of influencing sensitivity to any member of the cytokinin hormone family, including derivatives based thereon, or any purine-related signalling compound of plant origin, including derivatives based thereon. Additionally, signalling compounds derived from plant, fungi, bacteria or other pathogens or environmental stimuli signalling through a G protein, wherein the DNA of this invention influences sensitivity to these compounds in plants is also claimed. Derivatives of the compounds described above would include both natural or synthetic varieties.

Specifically, the invention relates to a gene having a nucleotide sequence either comprising the sequences shown in Fig. 1 or Fig. 2 or a substantially homologous sequence through degeneracy of the genetic code, including derivatives, mutants, or chimaeras or homologues capable of specific hybridization with the sequences shown in Fig. 1 or Fig. 2 and which still encode a G protein-coupled receptor homologue in plants.

As used in the present application, "substantial sequence homology" or "substantially homologous" is defined as a close structural relationship between nucleotides or

amino acids. For example, substantially homologous DNA sequences may be 60% homologous, preferably 80% and most preferably around 90 to 95% homologous, or more, as compared to a reference sequence and substantially homologous amino acid sequences may preferably be 35%, more preferably 50%, most preferably more than 50% homologous as compared to a reference sequence. Homology also includes a relationship wherein one or several subsequences of nucleotides or amino acids are missing, or subsequences with additional nucleotides or amino acids are interdispersed. A GPCR homologue would typically be defined as any protein which has a predicted structure characteristic of the GPCR superfamily (see: Strader *et al*, *Ann Rev. Biochem*, 63: 101-132 (1994)) and has significant amino acid sequence identity to GPCRs across all 7 putative transmembrane spanning regions or is a protein signalling through a G protein and bears a functional similarity with G protein-coupled receptors already known in vertebrates, invertebrates, arthropods, insects, nematodes, fungi, yeast, and viruses.

The degree of amino acid sequence identity may be calculated, for example, using a program such as "bestfit" (Smith and Waterman, *Advances in Applied Mathematics*, pp.482-489 (1981)) to find the best segment of similarity between any two sequences. The alignment is based on maximising the score achieved using a matrix of amino acid similarities, such as that described by Schwarz and Dayhof (*Atlas of Protein Sequence and Structure*, Dayhof, M.O., pp. 353-358 (1979)).

Where high degrees of sequence identity are present there may be relatively few differences in amino acid sequence. Thus, for example, they may be less than 20, less than

10, or even less than 5 differences in amino acid sequences.

As used herein, the term "specific hybridization" is defined as the formation of hybrids between a targeting transgene sequence (e.g., a polynucleotide of the invention which may include substitutions, deletions, and/or additions) and a specific target DNA sequence (e.g., a GPCR), wherein a labelled targeting transgene sequence preferentially hybridizes to the target such that, for example, a single band corresponding to a restriction fragment of a gene can be identified on a Southern blot of DNA prepared from cells using said labelled targeting transgene sequence as a probe. It is evident that optimal hybridization conditions will vary depending upon the sequence composition and length(s), and the experimental method selected by the practioner. Various guidelines may be used to select appropriate hybridization conditions (see Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual* (1989), 2nd. Ed., Cold Spring Harbor, N.Y. and Berger and Kimmel, *Methods in Enzymology*, Vol. 157, *Guide to Molecular Cloning Techniques* (1987), Academic Press, Inc., San Diego, Calif., which are incorporated herein by reference.

A fragment of a DNA sequence as used by this invention is defined as any portion of the *AtCGR1* gene or a homologue thereof which influences sensitivity to cytokinins or to purine-related signalling compounds, including any synthetic derivatives of both or which can influence sensitivity to any other signalling coumpound derived from plant, fungal, bacterial or pathogen origin, including environmental stimuli which signal through a G protein. Discovering suitable fragments would certainly

be within the skills of one in the art. For example, each of the 7 putative transmembrane spanning regions, specifically, those regions of the GPCR involved in interacting with the G-alpha subunit of G proteins (in particular, cytoplasmic loops 2 and 3, including the amino acid residues in the transmembrane regions near to the cytoplasmic face) are possible *AtGCR1* fragment candidates for influencing sensitivity to the above-described compounds.

It will also be well understood by those skilled in the art that there are numerous ways of influencing the activity and/or sensitivity of a GPCR or GPCR homologue, such as *AtGCR1*. For example, the sensitivity of many GPCR-mediated responses is modulated profoundly by reversible phosphorylation of Ser and Thr residues in the C terminal cytoplasmic region of the receptor by GPCR kinases (GRKs) (RJ Lefkowitz, *Nature Biotechnology* **14:283-286** (1996)). This phosphorylation increases the affinity of the receptor for β-arrestins which sterically hinder interaction between the GPCR and the G protein, thus desensitizing the response. A GPCR phosphatase reverses this process and then re-sensitizes the receptor. *AtGCR1* has the characteristic Ser residues and a Thr residue in the C terminal cytoplasmic region and thus would make a good target (i.e. fragment) for manipulating sensitivity.

It would also be within the skills of those in the art to determine which amino acids in *AtGCR1* (or homologues thereto) affects (i) ligand binding; (ii) coupling to G proteins and (iii) sensitization/desensitization. This involves random or directed mutagenesis or making chimaeric constructs using fragments from other GPCRs and

then screening the mutated/chimaeric receptors in suitable ligand-binding assays (for example, see: Kim *et al*, *JBC* **272**:2060-2068 (1997); Caterina *et al*, *JBC*, **269**: 1523-1532 (1994); Kim *et al*, *JBC*, **269**:28724-28731 (1994); Milne *et al*, *JBC*, **272**:2069-2076 (1997); and R.J. Lefkowitz, *Nature Biotechnology*, **14**:283-286 (1996)). This would enable the identification of groups of amino acids or even individual amino acids that could be used to manipulate sensitivity to cytokinin or signalling from *AtGCR1* or plant GPCR homologues.

This invention, therefore, also relates to the cDNA sequence of Fig. 2, wherein said cDNA sequence encodes for a GPCR homologue isolated from the family *Cruciferae*, or more specifically from the genus/species: *Arabidopsis thaliana* or any polypeptide having approximately 35%, preferably at least 50%, and most preferably more than 50% homology with the cDNA sequence of Fig. 2, said GPCR homologue signalling through a G protein in plants. A particular embodiment of this invention is that the GPCR homologue is capable of influencing sensitivity to cytokinins in plants. In another preferred embodiment of the invention, the isolated or recombinant DNA is in the form of a cDNA clone and said cDNA clone encodes for a polypeptide which is capable of influencing the sensitivity of a subject plant to the cytokinin, benzyladenine.

It is also an aspect of the present invention to provide a genetic construct comprising, a nucleotide sequence encoding a G protein-coupled receptor homologue, expression signals which include both promoter and terminator sequences and other regulatory sequences of the 3' and 5' untranslated regions and which are operably

linked to the coding DNA sequence so as to ensure the expression of the corresponding gene product in the respective host organism. For instance, the regulatory sequence may be inducible by use of a chemical. Suitable control sequences that are preferred within the scope of the invention are those comprising promoter and 5' and 3' untranslated regulatory sequences that are functional in plants. The regulatory sequence may also be obtained from either a cytokinin-inducible gene, cytokinin-regulated gene or a pathogenesis-related protein gene. Regulatory sequences in general may, independently, be derived from any source, such as, for example, virus, plant or bacterial genes. The promoters and regulatory sequences can be constitutive in nature or can be regulated in their patterns of expression. Such regulation may be temporal or spatial and include developmentally-regulated promoters, inducible promoters, tissue-preferential promoters or tissue-specific promoters. In particular, pith-specific promoters are desirable. Proteins may be optionally targeted to the vacuole or extracellularly using methods well known in the art. It is also within the scope of this invention to include chimaeric genetic constructs, wherein the chimaeric construct may comprise, for example, constructs in which domains or amino acids involved in either ligand binding, interaction with G proteins or receptor sensitivity are altered so as to confer either different ligand-binding characteristics, the ability to signal to a different (heterologous) G protein or to alter sensitivity to regulation.

In general, any promoter and any terminator capable of going about an induction of the expression of a coding DNA sequence (structural gene) may be used as a constituent of the gene sequence according to the

invention. The expression signal may promote continuous and stable expression of the gene. Especially suitable are expression signals originating from genes of plants or plant viruses. Examples of suitable promoters and terminators are those of a cauliflower mosaic virus gene or of homologous DNA sequences that still have the characteristic properties of the mentioned expression signals. Also suitable are bacterial expression signals, especially the expression signals of the nopaline synthase genes (nos) or the opine synthase genes (ocs) from the Ti-plasmids of *Agrobacterium tumifaciens*. Also to be mentioned here are, for example, ubiquitin promoters, actin promoters, histone promoters and tubulin promoters. Other suitable promoters are an amylase promoter (A-amylase promoter) and a ABA (abscisic acid) inducible promoter.

In a further aspect of the invention a promoter region may be used that is obtainable from the GPCR homologue sequence as described hereinbefore.

Within the scope of this invention, preference is given to the 35S and 19S expression signals of the CaMV genome or their homologues which can be isolated from the CaMV genome using molecular biological methods, as described for example in Maniatis *et al.*, and linked to the coding DNA sequence. Further preferred are expression signals that comprise tissue-preferential of tissue-specific promoters. As mentioned above, a developmentally regulated promoter can also be used. Of course, in the present invention, any promoter which is functional in the desired host plant can be used to direct the expression of an associated gene. In general, the plant G protein-coupled receptor homologue may be linked to the

promoter region in either a sense or antisense orientation.

It is also an aspect of the invention to provide transformed host cells comprising recombinant DNA encoding a G protein-coupled receptor in operable linkage with expression signals including promoter and termination sequences which permit expression of said DNA in the host cell.

The invention further comprises a genetic construct comprising at least a part of a reverse G protein-coupled receptor nucleotide sequence, in operable linkage with plant or fungal expression signals including promoter and termination sequences capable of causing the reverse sequence to express antisense mRNA within a plant. Additionally, the transformed host cells containing the antisense mRNA construct is also claimed in this invention. Therefore, antisense coding regions for the *AtGCR1* gene are also contemplated by this invention. Introducing the coding region of a gene in the reverse orientation to that found in nature can result in the down-regulation of the gene and hence the production of less or none of the gene product. The RNA transcribed from antisense DNA is capable of binding to, and destroying the function of, a sense RNA version of the sequence normally found in the cell thereby disrupting function. It is not crucial for antisense RNA solely to be transcribed at the time when the natural sense transcription product is being produced. Antisense RNA corresponding to some or all of the DNA encoding the G protein-coupled receptor may therefore be produced not only while the *AtGCR* gene or its equivalent is being expressed. Such antisense RNA may be expressed

constitutively, under the control of any appropriate promoter.

Another possible means of reducing expression is for example the use of ribozyme technology as described in EP-A-321 201 or WO 89/05852. A combination of antisense and ribozyme technology may also be used with the scope of the present invention for modifying cytokinin sensitivity.

According to a further aspect of the invention, therefore, there is provided antisense nucleic acid which includes a transcribable strand of DNA complimentary to at least part of the strand of DNA that is naturally transcribed in a gene encoding the isolated G protein-coupled receptor homologue in *Arabidopsis* or an equivalent protein in another member of the family *Cruciferae*. Antisense expression of whole or part of a plant GPCR homologue would be expected to decrease the level of GPCR homologue and render the plant less sensitive to the natural ligand. Sense over-expression of the entire GPCR homologue would elevate the level of the GPCR homologue, likely increasing the sensitivity to the ligand. Further, sense over-expression of cytoplasmic regions of the GPCR homologue would possibly constitutively inhibit G protein signalling (Hawes *et al.*, *J. Bio. Chem.* **269**:15775-85 (1994); Luttrell *et al.*, *Science* **259**:1453-57 (1993)).

In a further embodiment of this invention, methods such as using a transgenic plasmid sequence, screening a library by testing for binding activity with *AtGCR1* or any other suitable method known in the art for isolating homologues to *AtGCR1* or compounds which bind to *AtGCR1* or prevent binding to the natural ligand are also claimed under this invention. Standard techniques in recombinant

DNA technology can be used as part of the method, such as hybridisation using cDNA probes, polymerase chain reaction using degenerate primers, and restriction fragment length polymorphisms (RFLP). For instance, a method of preparing a recombinant DNA encoding a plant GPCR homologue may include preparing a genomic or a cDNA gene library by customary methods known to those skilled in the art. The basic methods of producing genomic or cDNA gene libraries are described in detail, for example, in Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1982), while information relating to the transfer and application of those methods to plant systems will be found, for example in Mohnen *et al.*, *EMBO Journal*, **4**:1631-1635 (1985). Genomic DNA and cDNA can be obtained in various ways. Genomic DNA, for example, can, using new methods, be extracted from suitable cells and purified. In the present invention, the starting material used for the production of cDNA is generally mRNA, which can be isolated from selected cells or tissues. The isolated mRNA can then be used in reverse transcription as the matrix for the production of corresponding cDNA. Such techniques are well-known to those skilled in the art.

Extracted and purified DNA preparations may be cleaved into fragments for subsequent cloning. Genomic DNA or cDNA to be cloned, may be fragmented to a size suitable for insertion into a cloning vector either mechanical shearing or, preferably, by cleavage with suitable restriction enzymes. Suitable cloning vectors which are already being used as a matter of routine for the production of genomic and/or cDNA gene libraries include, for example, phage vectors such as the λ charon phages,

or bacterial vectors, such as the *E. coli* plasmid pBR322. Further, suitable cloning vectors are known to those skilled in the art and may be obtained from commercial sources such as, for example, that contained in the "fast track" mRNA isolation kit obtainable from INVITROGEN or the λ gt11 cloning kit of Amersham. From gene libraries produced in this manner, suitable clones comprising the desired gene or parts thereof, can then be identified in a screening programme, for example with the aid of suitable oligonucleotide probes (probe molecule), and then isolated. Various methods are available for identifying suitable clones, for example, differential colony hybridisation or plaque hybridisation. Immunological detection methods based on identification of the specific translation products may also be used.

Provided the amino acid sequence of the gene to be isolated or at least parts of that sequence are known, a corresponding DNA sequence can be drawn up on the basis of that sequence information. On the basis of that information, it is thus possible to draw up all the nucleotide molecules that can be used as probe molecules for the identification and isolation of suitable clones by hybridising the probe molecules with genomic DNA or cDNA in one of the methods described above.

An alternative method of cloning genes is based on the construction of a gene library composed of expression vectors. In that method, analogously to the methods already described above, genomic DNA, but preferably cDNA, is first isolated from a cell or tissue capable of expressing a desired gene product -- in the present case a G protein-coupled receptor homologue, such as *AtGCR1* -- and then spliced into a suitable expression vector. The

resulting library can then be screened using suitable means, preferably using antibodies, and those clones selected which comprise the desired gene or at least part of that gene as an insert.

Alternatively, total DNA from the DNA library, preferably from the cDNA library, can be prepared and used as a template for a PCR reaction with primers representing low degeneracy portions of the desired amino acid sequence. Preferably, the primers used will generate PCR products that represent a significant portion of the nucleotide sequence. The PCR products can be further probed to determine if they correspond to a portion of *AtGCR1* using a synthetic oligonucleotide probe corresponding to an amino acid fragment sequence. The cDNA clones and PCR products prepared as described above or fragments thereof, may also be used as a hybridisation probe to identify further DNA sequences from a homologous or heterologous source organism encoding a similar G protein-coupled receptor homologue to *AtGCR1*, such as, for example, from fungi or a mono or dicotyledonous plant. They may also be used as a RFLP marker to determine, for example, the location of other G protein-coupled receptors or a closely linked receptor in the plant genome or for marker assisted breeding (EP-A-0306139; WO 89/07647). We believe that the transmembrane (TM) domains of *AtGCR1* would be a good targeted region for designing PCR primers to isolate new GPCR homologues from *Arabidopsis* and homologues from other genera as well as from plants outside *Cruciferae* (also known as the family *Brassicaceae*). This approach ahs been used to PCR clne numerous novel GPCRs and GCPR homologues from other organisms (Parmentieret *et al*, *Nature*, **355**:453-455 (1992) and Raming *et al*, *Nature*, **361**:353-356 (1993)). Thus,

using the methods described above, it is therefore possible to isolate other genes encoding G protein-coupled receptor homologues in plants and fungi.

In other related aspects, the invention features vectors which contain recombinant or isolated DNA and which are preferably capable of directing expression of the protein encoded by the *AtGCR1* DNA in a vector-containing cell; and cells containing such vectors. Preferably, such cells are stably transfected with such isolated DNA. Vectors for propagating a given sequence in a variety of host systems are well known and can readily be altered by one of skill in the art so that the vector will contain the G protein-coupled receptor homologue sequence and will be propagated in a desired host. Such vectors include plasmids and viruses and hosts include eukaryotic organisms and cells, for example yeast, insect, plant, mouse or human cells, and prokaryotic organisms, for example *E. coli* and *B. sutillus*.

Cloning vectors generally carry an origin of replication, especially in origin replication as capable of functioning in *E. coli*, in *Agrobacterium* or in both, and, in addition, specific genes that lead to phenotypic selection features in the transformed host cell, especially to resistance to antibiotics or to specific herbicides. The transformed vectors can be selected on the basis of those phenotypic markers after transformation in a host cell. The cloning vectors and the host cell transformed with these vectors are generally used to increase the number of copies of the constructs cloned therein. With an increased number of copies it is possible to isolate the vector carrying the chimeric gene construction and prepare it, for example,

for insertion of the chimeric gene sequence into a plant cell.

Especially suitable within the scope of the invention are so-called shuttle vectors, which can stably replicate not only in one, but in at least two different host organisms such as, for example, in *E. coli* and *Agrobacterium tumifaciens* in the absence of a suitable selection marker. Selectable phenotypic markers that may be used within the scope of this invention include, for example, resistance to ampicillin, tetracyclin, hygromycin, kanamycin, methotrexate, G418 and neomycin. But this, which is given by way of example, is not intended to limit the subject of the invention. Preferred, however, is a binary vector system.

Ultimately, DNA in accordance with the invention (whether a promoter plus the *AtGCR1* gene product or antisense DNA to the *AtGCR1* gene) will be introduced into plant cells, by suitable means. Thus, according to a further aspect of the invention, there is provided a plant cell comprising DNA in accordance with the invention as described above. Preferably, DNA is transformed into plant cells using a disarmed Ti-plasmid vector and carried by *Agrobacterium* by procedures known in the art, such as EP-A-0116718 and EP-A-0270822. Alternatively, the foreign DNA could be introduced directly into plant cells using an electrical discharge apparatus. This method is preferred where *Agrobacterium* is ineffective, for example where the recipient plant is monocotyledonous. Any other method that provides for the stable incorporation of the DNA within the nuclear DNA of any plant cell of any species would also be suitable. This includes species of plants which are not currently

capable of genetic transformation.

Additionally, the DNA in accordance with the invention may also contains a second gene (a "marker" gene) that enables a transformed plant containing the foreign DNA to be easily distinguished from other plants that do not contain the foreign DNA. Examples of such a marker gene include antibiotic resistance (Herrera-Estrella *et al*, 1983), herbicide resistance (EP-A-0242246) and glucuronidase (GUS) expression (EP-A-0344029). Expression of the marker gene is preferably controlled by a second promoter which allows expression in cells other than for the hereinbefore described G protein-coupled receptor homologue, thus allowing selection of cells or tissue containing the marker at any stage of regeneration of the plant. The preferred second promoter is derived from the gene which encodes the 35S subunit of Cauliflower Mosaic Virus (CaMV) coat protein. However, any other suitable second promoter could be used.

Another possible method of introducing genetic material into plant cells comprises, for example, bringing plant cells into contact with viruses or with *Agrobacterium* comprising the DNA to be introduced. This may be achieved by infecting sensitive plant cells or by co-cultivating protoplasts derived from plant cells. Within the scope of this invention, cauliflower mosaic virus (CaMV) may be used as a vector for the insertion of the G protein-coupled receptor DNA sequence according to the invention into a plant.

Another method of inserting the G protein-coupled receptor DNA sequence into a cell makes use of the infection of the plant cell with *Agrobacterium*

tumefaciens and/or *Agrobacterium rhizogenes*, which has previously been transformed with the gene construction. The transgenic plant cells are then cultured under suitable culture conditions known to those skilled in the art, so that they form shoots and roots and whole plants are then formed.

A further possible method of transforming plant material comprises mixed infection using both *Agrobacterium rhizogenes* and transformed *Agrobacterium tumefaciens* as described by Peder *et al.*, *Molecular Genetics* **202** 388 (1986) for the transformation of carrots.

The G protein-coupled receptor homologue DNA sequence according to the invention may also be transformed into suitable plant cells by means of, for example, the TI-plasmid of *Agrobacterium tumefaciens* or the RI-plasmid of *Agrobacterium rhizogenes*. The Ti-plasmid or RI-plasmid is transferred to the plant in the course of infection by *Agrobacterium* and integrated in stable manner into the plant genome.

Any tDNA containing vector can be transferred into plant cells and permit selection of the transformed cells is suitable for use within the scope of this invention, such as, for example, a shuttle vector that comprises the G protein-coupled receptor DNA sequence (*AtGCR1*) cloned in between the left border sequence and the right border sequence and that is capable of stable replication both in *E. coli* and *Agrobacterium tumefaciens*. Again, preference is given to a binary vector system, but is not limited to such a vector system.

Possible methods for the direct transfer of genetic

material into a plant cell comprise, for example, the treatment of protoplasts using procedures that likely modify the plasma membrane, for example, polyethylene glycol treatment, heat shock treatment or electroporation, or a combination of those procedures (Shillito *et al.*, *Biotechnology* 3 1099-1103 (1985)). The list of possible transformation methods given above by way of example is not claimed to be complete and is not intended to limit the subject matter or application of the invention in any way.

Although the examples and figures provided relate only to a GPCR homologue in *Arabidopsis*, those skilled in the art will readily be able to identify equivalent receptors from other members of the family *Cruciferae*. For instance, included within the specification as Fig. 1 is a fragment of a homologue of *AtGCR1* isolated from oilseed rape (*Brassica napus--BnGCR1*). The genomic DNA sequence for this homologue is given in Fig. 10. It would also be with the abilities of one skilled in the art to use the TM regions to design PCR primers to isolate and clone other GPCR homologues from plants. For instance, this method was followed and a homologue was isolated from Brassica olesacea (i.e. broccoli) (*BoGCR1*). This nucleotide sequence is represented in Fig. 15.

The present invention also comprises transgenic plant propagation material, selected from any of protoplasts, cells, calli, tissues, organs, seeds, embryos, ovules, zygotes, etc., and especially, whole and preferably phenotypically normal plants, that have been transformed by means of the processes described above and comprises the recombinant DNA according to the invention in expressible form, and of said transgenic plant material.

The process for production of transformed plant material,

including whole plants, thus essentially comprises: first isolating from a suitable source or synthesising by means of known processes a DNA sequence for a plant GPCR homologue as described herein; operably linking said DNA sequence in a 5' to a 3' direction to plant expression sequences as defined hereinbefore; transforming the construct of the above step into plant material by means of known processes in expressing it therein; screening of the plant material treated according to the third step for the presence of a DNA sequence to the G protein-coupled receptor described herein; and optionally, regenerating the plant material transformed according to the third step to a whole and preferably phenotypically normal plant and may include the desirable new trait or property.

Plants particularly desirable for use in this invention include, but are not limited to: vegetable crops; herbs; arable crops; pasture grass; pasture legumes, such as Medicago species; fruits or ornamental trees, such as apple, pear, plum, citrus fruit, and grape; forestry trees, biomass crop, such as willow or poplar; an oilseed crop, such as oilseed rape or sunflower; tobacco; cotton; sugar beet; and graminaceous monocots.

The expression "asexual or sexual progeny of transgenic plants" includes by definition, according to the invention, all mutants and variants obtainable by means of known processes, such as, for example, cell fusion or mutant selection and which still exhibit the characteristic properties of the initial transformed plant, together with all crossing and fusion products of the transformed plant material.

The proliferation material of transgenic plants is defined relative to the invention as any plant material

that may be propagated sexually *in vivo* or *in vitro*. Particularly preferred within the scope of the present invention are protoplasts, cells, calli, tissues, organs, seeds, embryos, egg cells, zygotes, together with any other propagating material obtained from transgenic plants.

A further aspect of the invention is the provision of an antibody raised against or at least a part of the amino acid sequence for the isolated G protein-coupled receptor of Figs 1 and 2. Such an antibody is useful in screening a cDNA library in suitable vectors derived from plant tissue RNA.

It is also an aspect of the present invention to provide a method for screening potential agonists or antagonists to the *AtGCR1* gene for use in designing agrochemicals which affect cytokinin-related responses. Such a method would include expressing the *AtGCR1* gene in a heterologous cell, such as yeast or even mammalian cells, and then testing a range of compounds, such as known cytokinins and cytokinin derivatives, for its binding affinity (or lack of binding) to *AtGCR1*.

It should also be appreciated by those skilled in the art that the *AtGCR1* gene may affect sensitivity to cytokinins *in planta* by signalling through compounds other than G proteins.

Reference is made in this application to the following figures:

Fig. 1 The genomic nucleotide sequence of *AtGCR1* aligned with the fragment of *BnGCR1*. Introns are indicated in lower case, dots indicate sequence identities and dashes indicate gaps introduced to

maximise the alignment. The line drawing indicates intron/exon organisation and relationship between *AtGCR1* (top) and *BnGCR1* (isolated from *Brassica napus*, bottom). Numbers above exons (solid boxes) and below introns (lines) indicate their length in nucleotides.

Fig. 2 Nucleotide and deduced amino acid sequence of *AtGCR1* cDNA. TM-spanning domains are indicated by solid lines above the amino acid sequence and the positions of introns within the coding region are indicated by inverted triangles. Primers are underlined and alternative polyadenylation sites are double underlined.

Fig. 3 Kyte-Doolittle hydrophilicity plot. Analysis of the amino acid sequence of the ORF of *AtGCR1* was performed using Peptide structure of EGCG(VMS) 8.0 using a window of 10 and results are plotted as a hydrophobicity index. Predicted TM helices are numbered 1-7. A similar result was obtained using a window of 7.

Fig. 4 Serpentine model of *AtGCR1* showing residues conserved in other GPCRs. This figure also depicts the amino acid sequence and the possible arrangement of α-helical regions, intracellular and extracellular loops and the N-, C-terminii of *AtGCR1*.

Fig. 5 represents a multiple sequence alignment between *AtGCR1*, CAR1 (from *Dictyostrelium discoideum* cAMP receptor), CTRI (human calcitonin receptor) and CRF3 (rat corticotrophin releasing factor receptor). The later three are known G-protein coupled receptors.

Fig. 6 Radial dendrogram showing relationship of *AtGCR1* with GPCRs. Sequences compared were: Family A, M64391 (OLF-R), P34969 (5HT7); Family B, L2332, L2333, X72304,

L25438, P35353 and X72305 (CRF-Rs), L19475, X78936, M77184 and M74445 (PTH-Rs), L00587, P30988, X69920, M74420, L14617, L14618, L13040, L13041 and X70658 (CT-Rs); Family E, A54813, A46390, P34907, P35352 and P13773 (CARs). No outgroups specified. Sequences of peptides indicated to be most similar to *AtGCR1* were aligned using CLUSTAL V and analysed using the PHYLIP suite of programs. PROTDIST was used to compare sequences by the Dayhoff PAM algorithm, NEIGHBOR was used for neighbour-joining cluster analysis and radial phylogenetic trees were drawn using Treeview for Windows. Editing the sequence alignments, by deleting regions of gap introductions, yielded essentially the same tree (not presented) suggesting that the topology of the tree is robust.

Fig. 7 Northern blot. Poly A$^+$ RNA (9 μg) from *Arabidopsis* seedlings was probed with labelled *AtGCR1* cDNA.

Fig. 8 RT-PCR on *Arabidopsis* total RNA. Using cDNA generated from poly A+ RNA isolated from light- (lane 1) and dark- (lane 2) grown 4 day seedlings. Amplimers used produced a 1 kb cDNA product. Lane 3 is a positive control using the cDNA clone as template. Using total RNA from whole plant (lane 4), leaves (lane 5) and stems (lane 6) from two-week-old plants. Lane 7 is a minus template control. Using stems (lane 8), leaves (lane9) and roots (lane 10) from seven-week-old plants. Track m contains size markers indicated in base pairs (bp). Amplimers used to roduce a 265 bp product from cDNA and a 392 bp product from genomic DNA traces of which are present in the total RNA.

Fig. 9 Southern blot. *Arabidopsis* DNA (10 μg/track) probed with *AtGCR1* cDNA. Lane 1, *Eco*RI; lane 2, *Bam*HI.

Fig. 10 represents the genomic nucleic acid sequence of the *AtGCR1* homologue, *BnGCR1*, isolated from *Brassica napus*.

Fig. 11 details alignment of two EST sequences compared with that of the *AtGCR1* cDNA sequence. The single long open reading frame is between bases 202-1180; nucleotides are numbered in relation to the sequence of *AtGCR1*.

Fig. 12 BA-inhibition of root growth. Seedlings germinated and grown five days on basal medium without additions and with a range of concentrations of BA. BARs indicate standard errors.

Fig. 13 BA inhibition of etiolation. Seedlings germinated and grown in the dark for five days on basal medium without additions and with a range of concentrations of BA. Bars indicate standard errors.

Fig. 14 Eight-week-old *Arabidopsis* plants. The plant on left is a vector-only control. It has numerous flowering stems. the plant on the right is an Anti 11, T4 having a single short primary flowering stem, several shorter secondary flowering stems and more extensive vegetative growth than the control plant.

Fig. 15 represents the genomic nucleic acid sequence of the *AtGCR1* homologue,(*BoGCR1*),isolated from *Brassica olesacea* (broccoli).

The following non-limiting example is provided as an illustration of the above-described invention:

EXAMPLE

I. Isolation of the first plant GPCR *(AtGCR1)*

Initially, we tried to isolate a GPCR homologue is *Arabidopsis* with degenerate amplimers, as used to clone odorant receptors[5]. This was not successful, hence, we looked for putative GPCRs in dbEST. Using free text and protein sequence searches at:

http://www2.ncbi.nlm.nih.gov/dbST/dbest_query.html; and
http://lenti.med.umn.edu/gst/MotifExplorer.html

we identified seven *Arabidopsis*, one rice and one pine EST with homology to GPCRs. We concentrated on ATTS2866 (330 bp) which showed homology to the *Dictyostelium discoideum* cAMP receptor CAR1. The other end of the clone (ATTS1113) was not similar to GPCRs. A BLAST search with ATTS2866 found a homologous EST (ID 376) with no similarity to GPCRs. By nested primer PCR, we found EST ID 376 overlapped, and lay 5' to, ATTS2866. We used primers based on the 5' end of EST ID 376 and the 3' end of ATTS1113 to generate the genomic clone *AtGCR1* (Fig. 1) and a 1281bp cDNA from *Arabidopsis*. To confirm that *AtGCR1* was not unique to *Arabidopsis*, degenerate primers based on the open reading frame (ORF) were used to isolate a homologue (*BnGCR1*) from *Brassica napus* var. Fido DNA (Fig. 1 and Fig. 10). The exons of *AtGCR1* and *BnGCR1* are 91% identical with 100% amino acid conservation over the region examined. The genes have similar intron/exon organization suggesting structural and functional conservation.

Using our Marathon RACE library, we obtained a full

length *AtGCR1* cDNA (Fig. 2). Upstream of the postulated initiating methionine are two in-frame stop codons. Heterogeneity in the lengths of the 3'-untranslated regions (Fig. 2) suggested multiple polyadenylation sites. The longest ORF encodes 326 amino acids.

Hydropathy analysis of the ORF revealed a hydrophobic N-terminus, seven 20 to 25 residue hydrophobic regions separated by hydrophillic domains and a hydrophillic C-terminus (Fig. 3). Analysis of *AtGCR1* using PHDhtp, and against TMbase using TMpred, suggested that the N-terminus is extracellular, that there are seven strong putative transmembrane (TM) domains connected by alternating intracellular and extracellular loops and that the C-terminus is intracellular. The distribution of charged amino acids is consistent with this proposed structure with 24 of the 31 Lys or Arg residues predicted to be located intracellularly. The protein sequence of >700 GPCRs is known and all have seven stretches of 20-28 hydrophobic amino acids each capable of forming a TM α-helix. Thus, GPCRs are thought to traverse the membrane seven times with associated extracellular and intracellular domains. The proposed structure of *AtGCR1* is characteristic of GPCRs (Fig. 3).

The amino acid sequence of *AtGCR1* has a number of residues that are conserved in GPCRs (Fig. 4). The majority of GPCRs contain cysteines in the second and third extracellular loops that are thought to form a disulfide bridge. Mutation of these affects ligand binding and receptor expression. *AtGCR1* has cysteines in the first and second extracellular loops that likely form a disulfide bridge. The second extracellular loop of *AtGCR1* has a potential N-linked glycosylation site,

consistent with many GPCRs. TM2 of *AtGCR1* contains the motif LAXXD which is highly conserved in family A GPCRs. The aspartate of this motif is one of the most highly conserved amino acids in GPCRs and is thought to be involved in transduction of agonist binding. At the boundary between TM3 and the second cytoplasmic loop, *AtGCR1* has an arginine conserved in virtually all GPCRs. Other conserved residues include tryptophan in TM4, the motif FXXP in TM5 and asparagine and tyrosine in TM7. In addition, *AtGCR1* has multiple serines and threonines in the C-terminus that may be sites of reversible phosphorylation and regulation by receptor kinases. For example, serine 303 in the *Dicteostelium* cAMP receptor, CAR1, is phosphorylated during agonist-induction and this serine is conserved in *AtGCR1*.

We compared the amino acid sequence of *AtGCR1* against Genbank, PIR, PDB and SwissProt databases using FASTA, BLAST and BEAUTY and against the GPCR database using FASTA. These analyses revealed sequence identities between *AtGCR1* and members of three of the six known GPCR families (Kolakowski, L.F., Jr. *Receptors and Channels*, 2: 1-7 (1994)) (Fig. 5). The plant GPCR showed highest similarity to all members of family F, [*Dicteostelium* cAMP receptors (CAR1-4)], significant similarity to family B [corticotrophin releasing factor receptors (CRF-R) and calcitonin receptors (CT-R)] and a number of serotonin (5HT7) and olfactory receptors of family A. A score table is presented comparing *AtGCR1* with the highest scoring representatives from these families (Table 1). Multiple sequence alignment of *AtGCR1* against three GPCRs with which it shows highest similarity showed strong collinear sequence similarity and good alignment of all seven putative TM regions (Fig. 5). Alignment of

AtGCR1 with the 5HT7 receptor (P34969) also indicated good collinear alignment over the seven TM domains (not presented). Phylogenetic analysis (Fig. 6) indicated that *AtGCR1* may form the basis of a new family of GPCRs.

Northern blot analysis indicated low levels of the *AtGCR1* transcript (Fig. 7). RT-PCR revealed expression in whole, two-week-old plants and in root, stem and leaves of two-week and seven-week plants (Fig. 8). It remains likely that there may be high level expression but within specific cells of each tissue. The *Arabidopsis* Gα-subunit gene GPA1 is expressed in a range of tissues during all stages of development of the plant.

Southern blot analysis indicates *AtGCR1* is a single copy gene (Fig. 9). *AtGCR1* cDNA was therefore used to screen the *Arabidopsis* CIC library in pYAC4. Two YAC clones hybridised to *AtGCR1*, one of which was anchored to the RFLP marker *m291b* which maps to chromosome 5 at 76.4cM.

II. Identification of the function for *AtGCR1*

To determine the function of *AtGCR1 in planta*, we introduced the cDNA in the antisense orientation. At the second generation (T2) six out of 24 antisense lines generated segregants with reduced cotyledon and leaf expansion and flowering stem production at the 7 leaf stage. We refer to this as a Dainty phenotype. A representative Anti 11 plant with a single T-DNA insert and Dainty phenotype was studied. PCR confirmed the antisense *AtGCR1* cDNA co-segregated with Dainty phenotype. The Dainty phenotype resembles the cytokinin-resistant mutant of *Arabidopsis* cyr1, located on chromosome 5, between 76.2 and 77.6cM. The similar phenotypes of *cyr1* and Anti 11 plants, combined with the

map location, led us to test the susceptibility of Anti 11 plants to cytokinins. The rationale for this was supported by the chemical similarities between cytokinins and cAMP, the ligand for the GPCRs to which *AtGCR1* shows the greatest similarity.

Anti 11, T3 seeds from a parent with a Dainty phenotype were plated on the cytokinin, benzyladenine (BA). Wild type *Arabidopsis* had roots 20±1.5mm long after five days' germination, which were reduced by 98% to 0.4±0.1mm on BA. *Arabidopsis* carrying the binary vector had roots 21.3±1.6mm long reduced by 96% to 0.8±0.3mm on BA. The Anti 11 seedlings had roots 15.5±1.1mm long which were, however, reduced by 54% to 7.1±0.3mm on BA. This suggested that antisense *AtGCR1* had reduced the sensitivity of the seedlings to BA.

The Dainty phenotype and reduced sensitivity to BA were stably inherited into the T4 generation. We then tested the response to a range of plant hormones (Table 2). BA-inhibition of root growth was significantly lower, over a range of concentrations of BA, in antisense ATGCR1-containing T4 plants compared with the control. Because cytokinin inhibition of root growth is thought to involve cytokinin-induction of ethylene which inhibits cell growth (Cary, A.J., Liu, W. & Howell, S.H. *Plant Physiol.* **107**: 1075-1082 (1995), we examined sensitivity to the ethylene-generating compound 1-aminocyclopropane-1-carboxylic acid (ACC). We found no difference between control and antisense plants (Table 2). Furthermore, inhibition of root growth by auxin was virtually identical in control and antisense plants (Table 2). Germination of both control and antisense plants was strongly suppressed by abscisic acid (10μM). When

control and antisense plants were germinated on gibberellin (5 μM), hyocotyl extension was comparable (not shown). Control and antisense plants showed normal etiolation when germinated and grown for five and seven days in the dark, indicating no difference in sensitivity to endogenous brassinosteroids (Hooley, R. *TIG* **12**: 281-283 (1996). If *AtGCR1* is playing an important role in cytokinin signal transduction we would expect that a reduced sensitivity to cytokinin would be apparent in other tissues of antisense *AtGCR1* plants. Cytokinins can overcome etiolation in *Arabidopsis* (Chory *et al.*, *Plant Physiol.* **104**: 339-347 (1994)) and we demonstrated that BA was significantly less effective at reducing hypocotyl elongation in dark-grown antisense *AtGCR1* seedlings compared with the control. This reduction in cytokinin sensitivity in shoot tissues is further supported by the pronounced apical dominance of Anti 11, T4 plants (Fig. 14). Thus, *AtGCR1* influences cytokinin sensitivity in both roots and shoots. It should be noted that the T4 generation of the Anti 11 plants displayed substantially delayed flowering and consequently an extended phase of vegetative growth. By suppressing flowering by prolonging vegetative growth would increase the yield of vegetables, particularly those that are prone to bolting (e.g. cabbage, lettuce, onion and other Allium sp, and sugar beet).

To determine the relationship of *AtGCR1* to *cyr1*, we amplified *AtGCR1* from *cyr1* seedling DNA. The amino acid sequence of the ORF from *cyr1* and CYR1 were identical and there were no differences at intron/exon splice sites. *AtGCR1* does not therefore appear to be involved in the *cyr1* mutation. CYR1 likely encodes a closely linked and possibly downstream component for the *AtGCR1* GPCR.

Table 1 Amino acid sequence similarity and identity values between *AtGCR1* and GPCRs

		% Similarity				
		AtGCR1	CRF-R	CT-R	CAR1	5HT7
% Identity	AtGCR1		46	47	53	45
	CRF-R	21		57	48	41
	CT-R	18	33		47	44
	CAR1	23	17	21		40
	5HT7	18	20	20	16	

The deduced amino acid sequence of *AtGCR1* was compared, using the GAP programme of EGCG 8.0, against the *Rattus Norvegicus* corticotropin releasing factor receptor 1 (CRF-R) (P35353) (24), human calcitonin receptor (CT-R) (P30988) (25), *Dictyostelium discoideum* cAMP receptor (CAR1) (P13773) (26) and the human 5-hydroxytryptamine receptor 7 (5HT7)(P34969)[16].

Table 2

Hormone	Mean root length (mm)		
	Vector control	Anti 11, T4	p value
None	11.9 (± 0.6)	11.0 (±0.5)	0.2507 (-)
BA	1.4 (±0.3)'	3.9 (±0.4)	<0.0001 (***)
IAA	1.7 (±0.5)	1.4 (±0.2)	0.4623 (-)
ACC	1.8 ±(0.3)	1.8 (±0.3)	1.0000 (-)

Root lengths were measured four days post-germination of *Arabidopsis* seedlings (mean of >20). P values were determined by unpaired two-tailed t test. (-); not significant, (***); extremely significant.
BA, benzyladenine (6 μM); IAA, indole-3-acetic acid (10 μM); ACC, 1-aminocyclopropane-1-carboxylic acid (10 μM).

III. Specific methodology used for isolation, cloning and identification

A. Plant material. *Arabidopsis thaliana* (Colombia) was germinated on tissue culture plates in Murashige and Skoog Basal Medium (Sigma) with 1% agarose and 1.5% sucrose, and maintained in containment rooms. Plants for seed production were grown under transgenic greenhouse conditions.

To generate transgenic lines, the flowering parts of five-week-old plants were vacuum infiltrated[31] with *Agrobacterium tumefaciens* (GV3101) carrying the plant binary transformation vector, pLARS120. The *AtGCR1* cDNA

sequence was cloned into the vector in the antisense orientation, under the control of a 35S cauliflower mosaic virus promoter cassette. After 4 - 6 weeks, seeds were harvested, transformants selected on plates with kanamycin (50 μg/ml) and grown to seed. The selection was repeated to identify those with a segregation ratio of 3 resistant to 1 sensitive plant (one copy of the T-DNA inserted).

Seeds of the mutant *cyr1* were obtained from the *Arabidopsis* Biological Resource Center, Ohio USA. These were germinated and the homozygous plants distinguished from the heterozygotes and wild type by virtue of their failure to thrive. DNA was prepared after seven days' germination on tissue culture plates.

B. Preparation of polyA⁺ RNA. PolyA⁺ RNA was isolated from seven-day-old etiolated *Arabidopsis* by phenol/chloroform extraction and oligo dT cellulose (Pharmacia) chromatography.

C. Rapid amplification of cDNA ends (RACE) library construction. A Marathon cDNA RACE library (Clontech) was constructed using 1 μg of polyA⁺ RNA according to manufacturer's instructions.

D. RT-PCR. Total RNA was isolated from whole plant, roots, leaves and stems (0.1 g) using the RNeasy protocol (Qiagen). Reverse transcription of total RNA (5 μg) was carried out with Bulk First Strand Reaction Mix (5 μl) from a First-Strand cDNA Synthesis Kit (Pharmacia). Aliquots (5 μl) were used in PCR. *Taq* DNA Polymerase (Gibco BRL) was used for the PCR in the Basic Protocol suggested by the manufacturer. Cycling parameters were

94°C; 55°C; 72°C each at 1 min for 30 cycles using a Perkin Elmer 480 DNA Thermal Cycler.

E. Genomic DNA extraction. DNA was extracted from leaves (0.1 g) using the Easy-DNA Kit (Invitrogen). Aliquots (100 ng) were used in PCR under the same conditions as for RT-PCR but with 2 min at the annealing cycle and 50°C annealing for *B. napus*.

F. Subcloning and sequence analysis. PCR products were subcloned into pCRII (Invitrogen). Plasmids were purified using Plasmid-Tip100 columns (Qiagen). DNA was sequenced using a T7 Sequencing Kit (Pharmacia) and by automated sequencing (Dr J. Hancock, Bristol).

G. Northern and Southern blots. PolyA$^+$ RNA (9 μg) from seven-day-old etiolated seedlings was run on formaldehyde-denaturing 1% agarose gels and transferred to Hybond N+ (Amersham), hybridised and washed according to manufacturer's instructions. Enzyme-restricted *Arabidopsis* DNA immobilised on Hybond N (Amersham) was hybridised according to the manufacturer's instructions. Probes were generated using *AtGCR1* cDNA insert and were purified on agarose gels by the Qiaex II procedure (Qiagen). Labelling was with [α-^{32}P]dCTP using an Oligolabelling Kit (Pharmacia).

H. Mapping on YAC clones. The CIC library in YAC clones immobilised on membranes was a kind gift of Dr C. Dean (John Innes Centre) and was probed with the full length cDNA clone by Dr K. Edwards (IACR-Long Ashton).

Claims

1. A recombinant or isolated DNA or a fragment thereof, said DNA or fragment thereof encoding for a polypeptide in plants having homology to a G protein-coupled receptor.

2. A recombinant or isolated DNA as claimed in claim 1, wherein said polypeptide is capable of influencing sensitivity to cytokinins or any derivatives thereof, to purine-related signalling compounds or any derivatives thereof, or to signalling compounds isolated from plants, fungi, bacteria, or other pathogens or environmental stimuli signalling through a G protein, in plants.

3. A recombinant or isolated DNA as claimed in claim 2, wherein the cytokinin is benzyladenine.

4. A recombinant or isolated DNA of any one of claims 1, 2 or 3, wherein said DNA comprises the *AtCGR1* sequence of Fig. 1 or is a DNA having substantial sequence homology thereto or a fragment thereof.

5. A recombinant or isolated DNA of any one of claims 1 to 4, wherein said DNA is a cDNA comprising the sequence of Fig. 2 or is a cDNA having substantial sequence homology thereto or a fragment thereof.

6. A recombinant or isolated DNA as claimed in claim 5, wherein said DNA sequence encodes an amino acid sequence of Fig. 2 or is an amino acid sequence having substantial sequence identity with the amino acid sequence of Fig. 2 due to the degeneracy of the genetic code.

7. A recombinant or isolated DNA of claims 1 to 6, wherein said DNA encodes for a G protein-coupled receptor homologue capable of influencing sensitivity to cytokinins or derivatives thereof or to purine-related signalling compounds or derivatives thereof, in plants, preferably in monocotyledonous plants and most preferably in dicotyledonous plants.

8. A recombinant or isolated DNA as claimed in claims 1 to 7, wherein said G protein-coupled receptor homologue influences sensitivity to cytokinins or derivatives thereof or to purine-related signalling compounds, or any derivatives thereof, in plants of the family *Cruciferae*.

9. A recombinant or isolated DNA as claimed in claims 1 to 8, or a fragment thereof, wherein said G protein-coupled receptor homologue influences sensitivity to cytokinins or derivatives thereof or to purine-related signalling compounds or derivatives thereof, in *Arabidopsis thaliana* or a protein having at least 35%, preferably at least 50%, and most preferably at least more than 50% homology therewith.

10. A recombinant or isolated DNA as claimed in claims 1-3 and 7-8, wherein said DNA comprises either a *BnGCR1* sequence of Fig. 10 or *BoGCR1* of Fig. 15 or is a nucleotide sequence having substantial homology thereto or a fragment thereof.

11. A recombinant or isolated DNA as claimed in claim 10, or a fragment thereof, wherein said G protein-coupled receptor homologue influences sensitivity to cytokinins in *Brassica napus* or *Brassica oleracea* or a protein having at least 35%, preferably at least 50%, and most

preferably at least more than 50% homology therewith.

12. A recombinant or isolated DNA as claimed in any one of claims 1 to 11, comprising a nucleotide sequence corresponding to the open reading frame of the sequences of Fig. 1, or an equivalent sequence through the degeneracy of the genetic code, including derivatives capable of hybridizing with the sequences of Fig. 1 and which encode for a polypeptide in plants having substantial sequence homology to a G protein-coupled receptor.

13. A recombinant or isolated DNA as claimed in claim 12, wherein said peptide with homology to a G protein-coupled receptor is capable of influencing sensitivity to cytokinins or derivatives thereof, to purine-related signalling compounds, or any derivatives thereof, or to signalling compounds compounds isolated from plants, fungi, bacteria or other pathogens or environmental stimuli signalling through a G protein, in plants.

14. A method of isolating a DNA as claimed in claims 1 to 13, said method comprising preparing a cDNA library from a suitable source organism and screening said library by means of one of the conventionally applied screening systems.

15. The method as claimed in claim 14, wherein the screening is by means of an antibody against *AtGCR1* or an antibody against part of its amino acid sequence or by testing for activity which is characteristic of *AtGCR1*.

16. The method as claimed in claim 14, wherein the screening is by means of an antibody against *BnGCR1* or

BoGCR1 or an antibody against part of its amino acid sequence or by testing for activity which is characteristic of *BnGCR1* or *BoGCR1*.

17. An isolated G protein-coupled receptor homologue in plants, wherein said homologue signals through a G protein.

18. An isolated G protein-coupled receptor homologue of claim 17, wherein said homologue is capable of influencing sensitivity to cytokinins or deriviatives thereof or to purine-related signalling compounds or derivatives thereof, in plants, preferably in monocotyledonous plants and most preferably in dicotyledonous plants.

19. An isolated G protein-coupled receptor homologue as claimed in claim 17 or claim 18, wherein said homologue influences sensitivity to cytokinins or derivatives thereof or to purine-related signalling compounds or derivatives thereof, or to signalling compounds isolated from plants, fungi, bacteria or other pathogens or environmental stimuli signalling through a G protein in plants of the family *Cruciferea*.

20. An isolated G protein-coupled receptor homologue as claimed in any one of claims 17, 18, or 19, wherein said homologue is obtainable from *Arabidopsis thaliana* or is a protein having at least 35%, preferably at least 50% or most preferably more than 50% homology with a native *Arabidopsis thaliana* G protein-coupled receptor homologue.

21. An isolated G protein-coupled receptor homologue as

claimed in any one of claims 17, 18 or 19, wherein said homologue is obtainable from *Brassica napus* or *Brassica oleracea* or is a protein having at least 35%, preferably at least 50% or most preferably more than 50% homology with a native *Brassica napus* or *Brassica oleracea* G protein-coupled receptor homologue.

22. An isolated G protein-coupled receptor homologue comprising an amino acid sequence encoding a polypeptide having at least 35%, preferably at least 50% and most preferably more than 50% sequence identity with the amino acid sequence of Fig. 2, wherein said polypeptide encodes a G protein-coupled receptor homologue in plants.

23. An isolated G protein-coupled receptor homologue as claimed in claims 22, wherein said polypeptide is capable of influencing sensitivity to cytokinins or derivatives thereof or to purine-related signalling compounds, or any derivatives thereof, in plants.

24. An isolated G protein-coupled receptor homologue as claimed in claim 23, wherein the cytokinin is benzyladenine.

25. A method of isolating the G protein-coupled receptor as claimed in any one of claims 17 to 24, wherein said method comprises expressing in a suitable host organism a DNA sequence as claimed in any one of claims 1 to 13.

26. The method as claimed in claim 25, wherein said host organism is a transformed host cell.

27. A genetic construct comprising a DNA as claimed in any one of claims 1 to 13, said DNA operably linked to a

promoter which naturally drives the expression of a gene encoding a G protein-coupled receptor homologue in plants and to a termination sequence.

28. A genetic construct comprising an antisense G protein-coupled receptor homologue nucleotide sequence or fragment thereof, wherein said antisense nucleotide sequence is operably linked to promoter and termination sequences which drive the expression of antisense mRNA in plants.

29. A genetic construct as claimed in claim 27 or 28, wherein the promoter is any one of an inducible promoter, tissue-preferential promoter, or tissue-specific promoter.

30. A genetic construct as claimed in claim 29, wherein said tissue-specific promoter is a pith-specific promoter.

31. A genetic construct as claimed in any one of claims 27 to 30, further comprising a regulatory sequence of both the 5' and 3' untranslated regions.

32. A genetic construct as claimed in claim 31, wherein the regulatory sequence is inducible by a chemical.

33. A genetic construct as claimed in 32, wherein the regulatory sequence is obtainable from either a cytokinin-inducible gene, cytokinin-regulated gene or a pathogenesis-related protein gene.

34. A genetic construct as claimed in claim 33, wherein the 3' transcription regulation signals are derived from

the Cauliflower Mosaic Virus 35S gene.

35. A genetic construct as claimed in any one of claims 27 to 34, further comprising a marker sequence which enables a plant transformed with the DNA of any one of claims 1 to 13 to be distinguished from plants not so transformed.

36. A genetic construct as claimed in claim 35, wherein the marker sequence confers antibiotic or herbicide resistance.

37. A genetic construct as claimed in claim 36, wherein the marker sequence is under the control of a second promoter.

38. A genetic construct as claimed in claim 37, wherein the second promoter is derived from a Cauliflower Mosaic Virus (CaMV) 35S gene.

39. A host cell transformed with the genetic constructs of any one of claims 27 to 38.

40. A host cell transformed with the DNA as claimed in any one of claims 1 to 13.

41. A host cell as claimed in claim 39 or 40, wherein said host cell is of bacterial or plant origin.

42. A plant, a part of said plant, or propagating material from a plant comprising plant cells transformed as claimed in claim 39 or claim 40.

43. A plant, a part of said plant, or propagating

material from said plant as claimed in claim 42, wherein said plant is any one of a vegetable crop, a herb, an arable crop, a pasture grass, a pasture legume, a fruit or ornamental tree, a forestry tree, a biomass crop, an oilseed crop, tobacco, cotton or sugar beet.

44. A plant as claimed in claim 43, wherein said plant is a monocotyledonous plant, preferably a graminaceous monocot.

45. A method of isolating a G protein-coupled receptor homologue in plants, said method comprising preparing a cDNA or a genomic library from a suitable source organism and screening said library using a hybridisation probe comprising a DNA as claimed in any one of claims 1 to 13.

46. An isolated DNA sequence encoding a G protein-coupled receptor homologue in plants isolated by the method as claimed in claim 45.

47. A method for screening potential agonists or antagonists to a G protein-coupled receptor homologue in plants, said method comprising expressing the DNA as claimed in any one of claim 1 to 13 in a heterologous cell and then testing a desired compound for binding affinity (or lack of binding affinity) with the polypeptide encoded by said DNA.

```
AtGCR1  CGACGAACAC GAACAGCGGA AATCGTCAAT TCAATCTCTC AGATCAGTTT TGGAATCACA
AtGCR1  TATTACGGCG ATTAACACCG GCGCTTCGAG TCTCTCATTC GTCGGCTCAG CCTTCATCGT
AtGCR1  TGCTCTCTCT gtaagtttcc cgttgttgcc tttcgctgat tgtgctagat ctaggattat
AtGCR1  cgttaagttc cattgcgttc tgagatgagt agtgagggtt cgagctctat tgctgactaa
AtGCR1  gtttatctac tactattggt tctggtgaat cttgttgaga ttattttgat aagatgattg
BnGCR1
AtGCR1  ACTACTCATT TCTTCTGCGT TGCTTCGTTC TTGTGGACAA CTACAATTGC TTTCACTCTT
BnGCR1  .....A.... .T..T..... ......C... .......... .C..T..... ..........
AtGCR1  TATGTATGGG gtatgtaaaa tgatactcgt gggtttcggt tctcaattgt tactatatcc
BnGCR1  .....T.... ...g..g... a..------.a a..a.a.tc. .t...ga.t. aga.cac..-
AtGCR1  ttttagttac ttttctcagG GACTTCCTTG GTTGTGACTG TCATACGTTC TTTTGGTAAC
BnGCR1  --------.t c..ggg.... A.......... .......... .......... .........T
AtGCR1  gagaatgctt agttgagcat aaagatttca atgcctatca aaaggcattt gaacttttt
BnGCR1  a.t...ct.. .c..t....a ..c... --- ---------- ---------- ----------
AtGCR1  tatcacctct tgaattttgc agGCTGTTCA TTTTTTAACT TTCTACGCTC CTCTTTGGGG
BnGCR1  .c.ttttctc ca.c..a... .......... ...C.....C .....T.... ..........
AtGCR1  ACGTgtatgt aactctctaa tctctaataa cattgtcttc tgaaagtagt tcacaaacgt
BnGCR1  .......... ..t.------ ---------- a........t .....a..t. cacat...c.
AtGCR1  TGTCAGACCG AGTCGATCAA TTTGATAATA GAGCAGAGTT AAAGgtct-- ---------g
BnGCR1  .......... ...G.....G .......... .......... ......t.tt ttcttcttct
AtGCR1  cactgcttgt ttcctgaaac cacagGTGTT GAACAGATGG GGATACTATC CACTCATTCT
BnGCR1  t.a....... .......... .......... ....C.T... ........C. ..........
AtGCR1  TAAGATCTTC TGGCTCTCAG TTCTTGACGT TGGGACAGCT GCACTAATGg tagcaaacct
AtGCR1  tctgaatgtt gtattttcag GGCTTGTTCA ATTCAATAGC CTATGGTTTC AACAGCTCAG
AtGCR1  tatattattc ccaataatca atgaagcttg atttttatga aacccttggt ctgctagATT
AtGCR1  TCTACATCAG CAACAACAAC AGCGAAGCGA AATGGTATCA CTCAAGACCG AGGACCAGCA
AtGCR1  CGTTTGGATG GAGAGATTGg taagtttcct ctagattttt ttgtctttaa tttccgttta
AtGCR1  CACAACAGGT TGTCAATGTA TATGAAACAT TCTTTGGGAA CACGAACTTA ACAATTTATT
AtGCR1  TTGGAGTTCT TGGACGACA  2283
```

FIG. 1(I)

```
TATAACAATG TCGGCGGTTC TCACAGCCGG CGGAGGCTTA ACTGCCGGGG ATCGCAGCAT  120

TCTCTGCTAC TGCCTCTTCA AAGAACTTCG AAAATTCTCT TTCAAGCTCG TCTTCTACCT  240

gtactgtttt gcttccttct tcttcgatta ttctcactgt gaattgttac atcgctaaga  360

tggccatgtt tttaattgtt gcagGATATG CTTTGCAGTT TCTTCCTAAT CGTTGGgtat  480

ttacatcttg atcactgtag GGATCCTTCA AAGGGGTTCA TTTGTTACGC ACAAGGCTAC  600
                                                              ..T

CATCGTACTG TTGTCAAGCA TAAAACTGAT GTGGAGGATT TGGAAGCAAT GTTTCATTTG  720
..C..C..C. .C........ ...G...... .....A.... .......C.. ......C...

acctagggtt aaggattaga atgtgttagt atcttctttg gtgatttagt aagactctga  840
---------- -..tt.g... .....c..aa g.g..ta.ac a.ccaaatcc .---------

AACCACTCAC ATTTGGGGCC ATGGTGCTGG ACGCAAACTG GTCTTAAGGG AAAGgcaagt  960
........T. .......... T......... ..T....... .......A.. .....t.caa

gttgcaacgc tgggccagat gaatgtagac tagtatttta ttcttcgtta ctggaactca 1080
---------- ---------- ---------- -----gg... c.ac.tac.. .ct..gtc.t

AGCCATTCTT TACAATGGGT TTACTTACTT CCAAGTGATA CGGATGCTAA GAAATGCTAG 1200
T........C ........C. ....A..... .......... .......... ....C.....

tactgataca cttggctgtg tgctgaaatg gcaatgtt-a atttcagATG GCAGTTGGAA 1319
c.t.tcg... ....t..a.c .a....c.c. .tgg....a. .......... ..G.......

tctttattgc caatactagt tctgtatcat gtggctg-ga t---ttcatc attaaaacct 1424
...a...... t.....-ca. .......at. a..t...act .tgg...c.a ga.t......

AATAGGATCA TGGGCATTCG GCACTATTAA CCGTATCCAT GATTTCATCG AGCCAGGGCA 1544
.......... .......... .T.....C.. ......T..C ..C..T.... .A...

atcaaataaa taagttataa aattcgccat agatcatctt tgctcttccc gagtcgatgt 1664

TGCGTCGAGC AATCCATGAG AGACTGGAGC Tgtaagtcac caccgatctc tttggtttgc 1784

CTTGCCAGAA CGGCTATATC GATGGCTTCC AAGCAATTTC AGACCAAAAA ACCATCTGAT 1904

ATGACACTAA CTTTCAACTA ATACATTGGA CCGATAACAA GAAGGCGGCG ATGATAATAG 2024

catggatcag taagtgtgtt ttgtttcgtg tttctctctt agATATGGTG AAGGGGGGTG 2144

TATTTTTGTA TGAACTTTTT TATTGTGAGG TCTTCTGATC TTGATTTGAA ATTAACGAAC 2264
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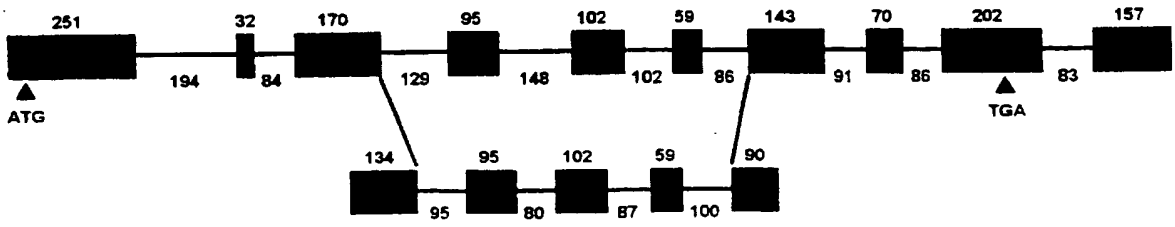

FIG. 1(II)

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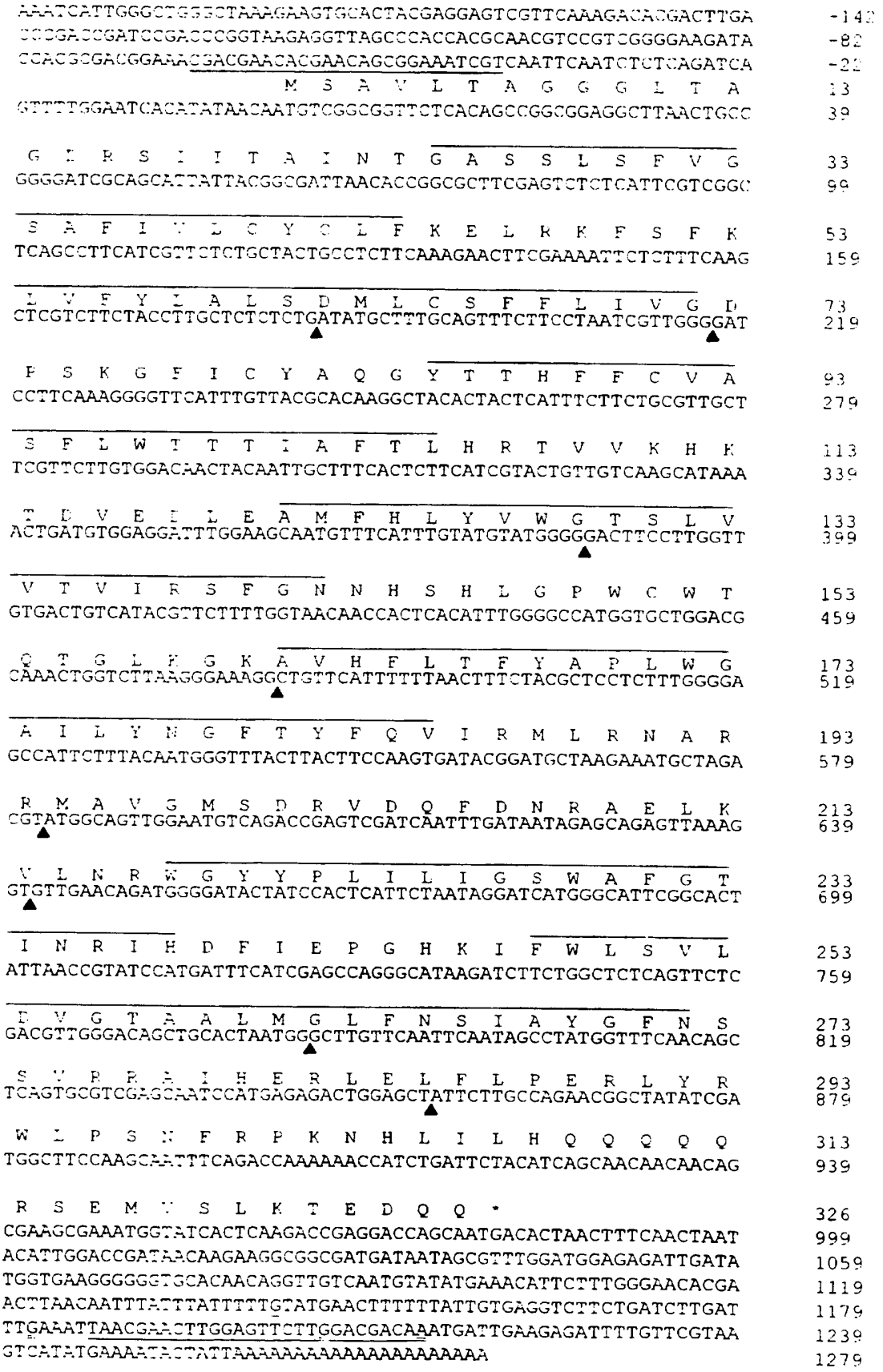


FIG. 2

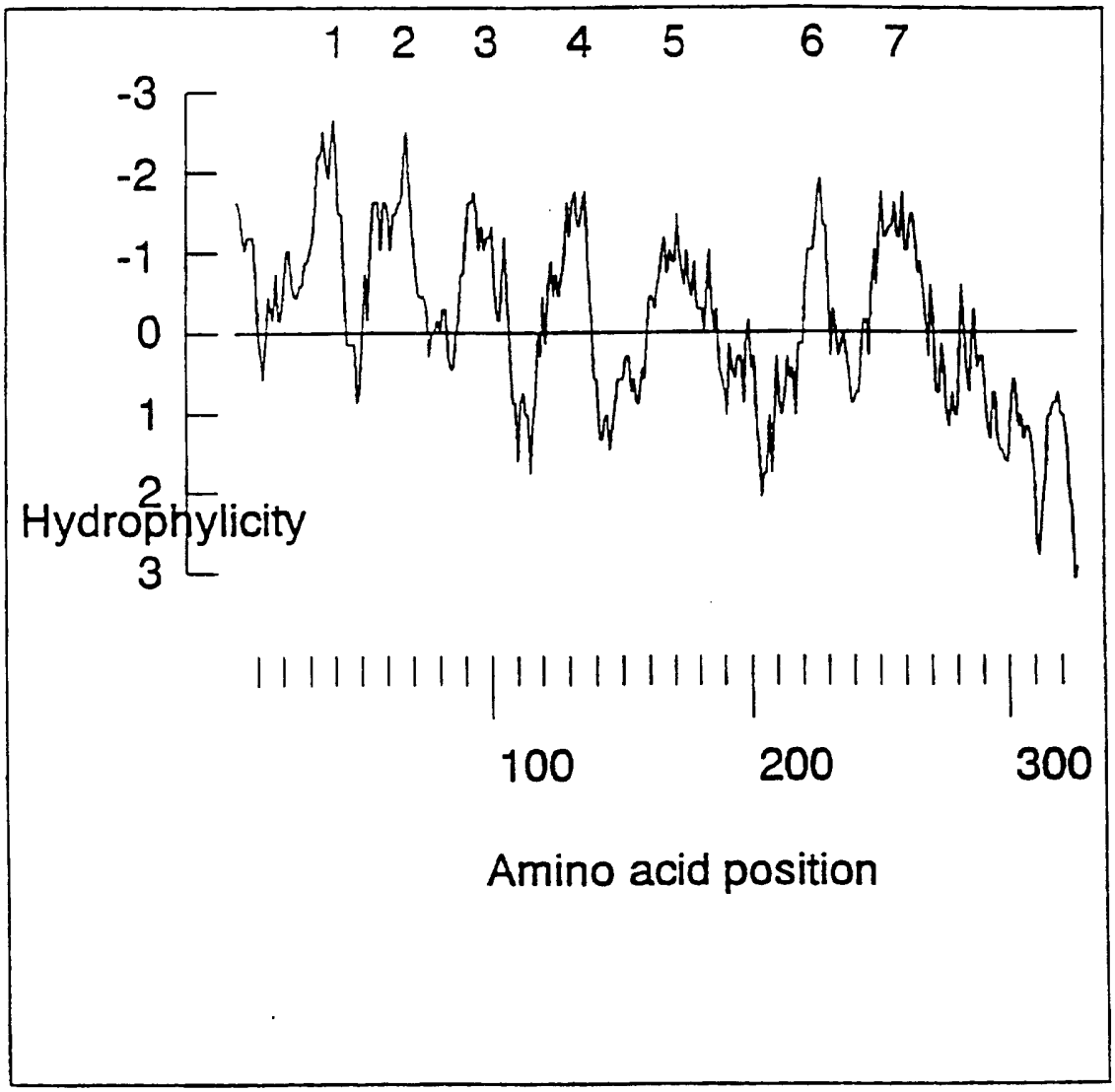
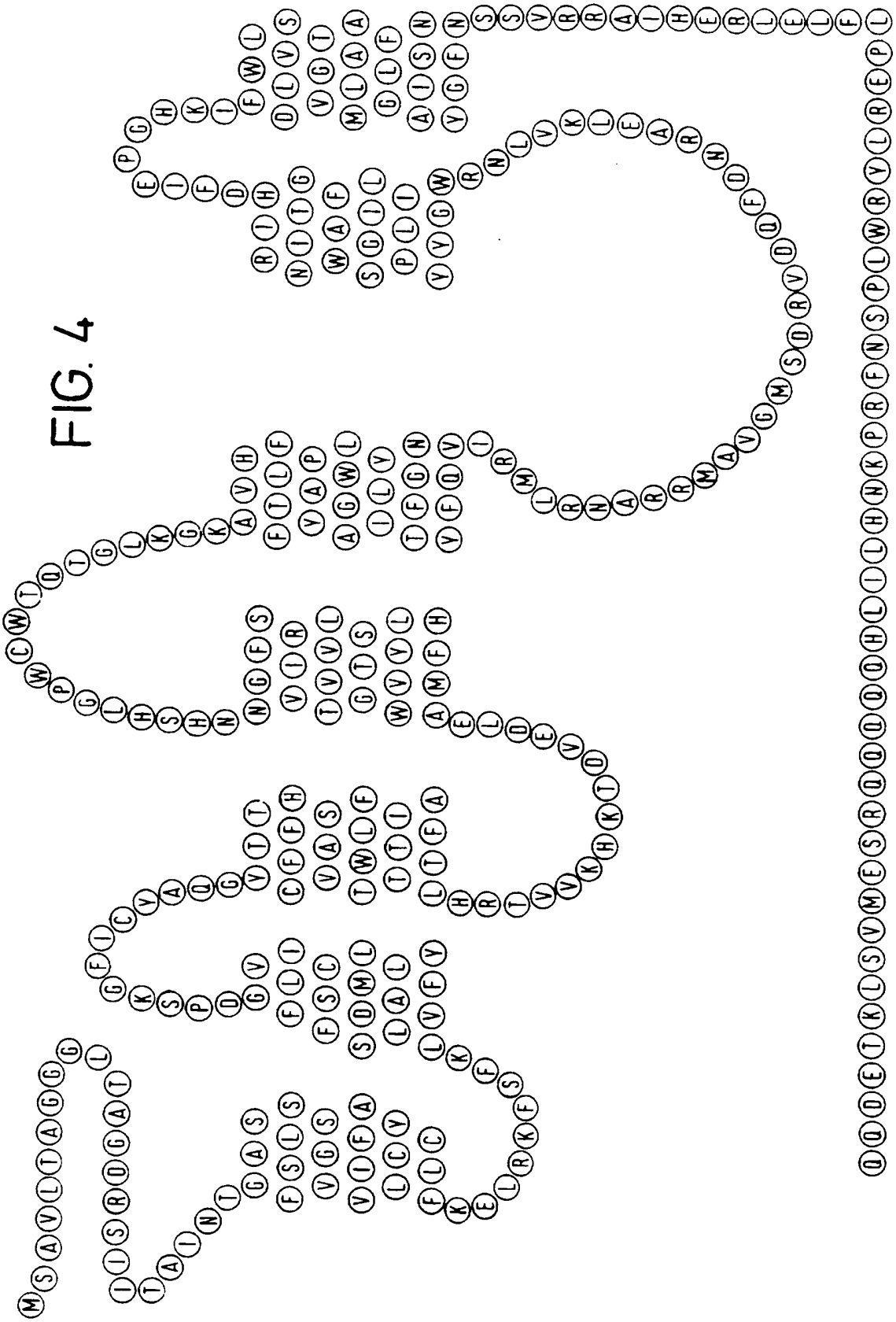


FIG. 3

FIG. 4



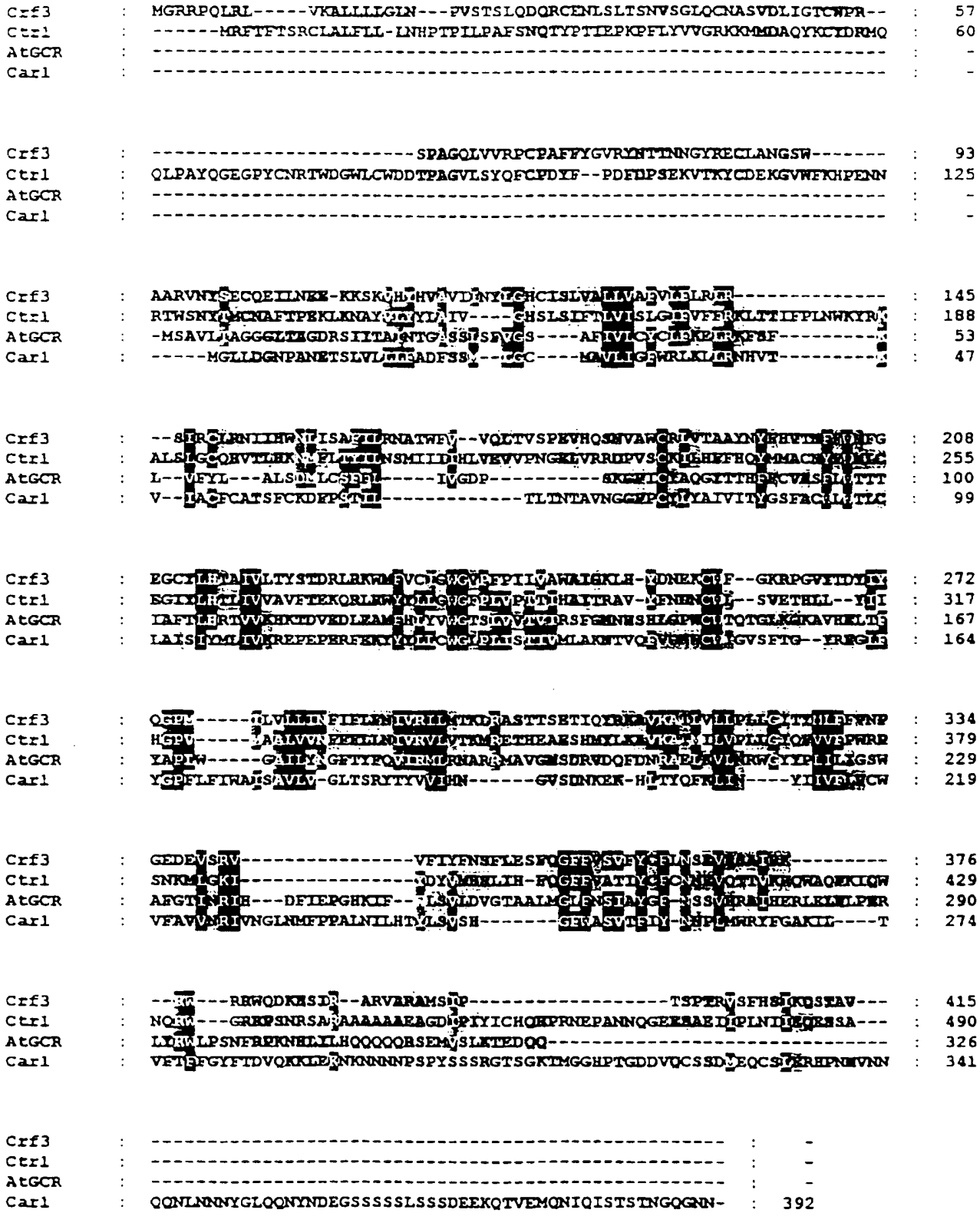


FIG. 5

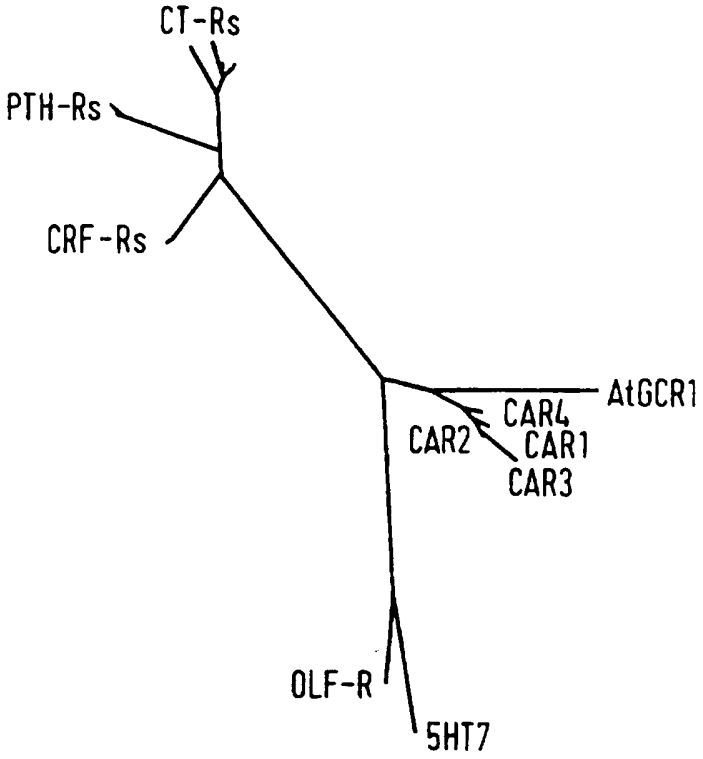


FIG. 6

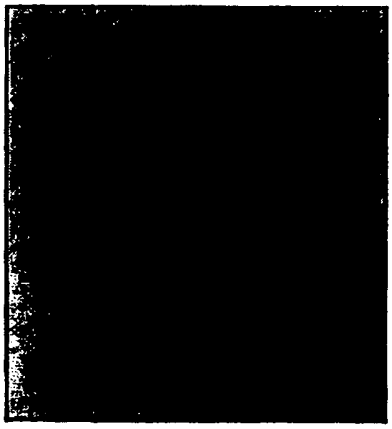


FIG. 7

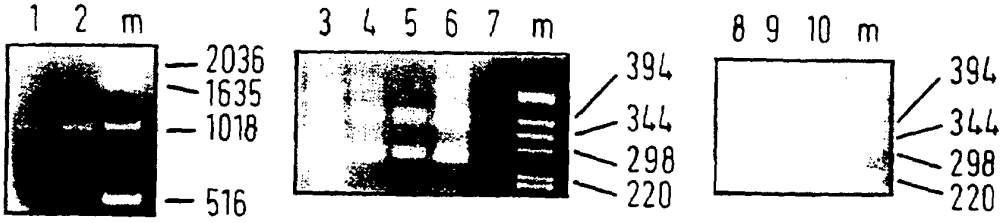


FIG. 8

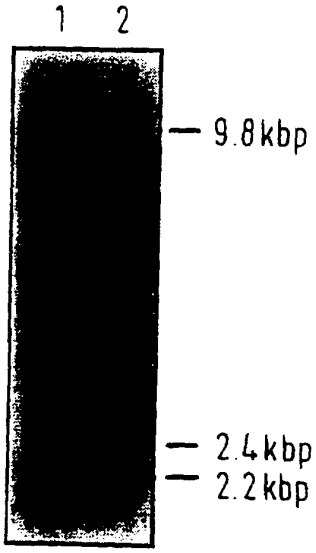


FIG. 9

```
  1  TATACTACAC ATTTTTTTTG CGTTGCTTCC TTCTTGTGGA CAACCACTAT
 51  TGCTTTCACT CTTCACCGCA CCGTCGTCAA GCATAAGACT GATGTGGAAG
101  ATTTGGAAGC CATGTTTCAC TTGTATGTTT GGGGTAGGTG AAAAGAGAAG
151  GATACTCTTT TCAGATTTAG ATCACTCAGT TTGAGAATGT GCTAAACTGT
201  TTATACATCC AAATCCAATC TTGGGCAGGA ACTTCCTTGG TTGTGACTGT
251  CATACGTTCT TTTGGTAATA ACCACTCTCA TTTGGGGCCT TGGTGCTGGA
301  CTCAAACTGG TCTTAAAGGA AAGGTACAAA ATAATCTTTA CTTTAGCAAA
351  ACGATTGGTT ACTACTTACT ACCTGAGTCC TTCTTTTTCT CCAACTTATG
401  CAGGCTGTTC ATTTCTTAAC CTTCTATGCT CCTCTTTGGG GTGCCATTCT
451  CTACAATGGC TTTACATACT TCCAAGTGAT ACGGATGCTA AGAAACGCTA
501  GACGTGTATG TAATTAATTG TCTTTTGAAA ATATTCACAT AACCTCATTT
551  CGACACTTGT CTATCTACTG ACACGGTGGT GTTAAATTTC AGATGGCGGT
601  TGGAATGTCA GACCGAGTGG ATCAGTTTGA TAATAGAGCA GAGTTAAAGG
651  TTTTTTTCTT CTTCTTCTAT ATTGCTAATA CCATTCTGTA TATTATGTCT
701  GACTTTGGTT CCTAGATTAA ACCTTAATGC TTGTTTCCTG AAACCACAGG
751  TGTTGAACCG TTGGGGATAC TACCCACTCA TTCTAATAGG ATCATGGGCA
801  TTCGGTACTA TCAACCGTAT TCACGACTTT ATCGAACCA
```

FIG. 10

FIG. 11

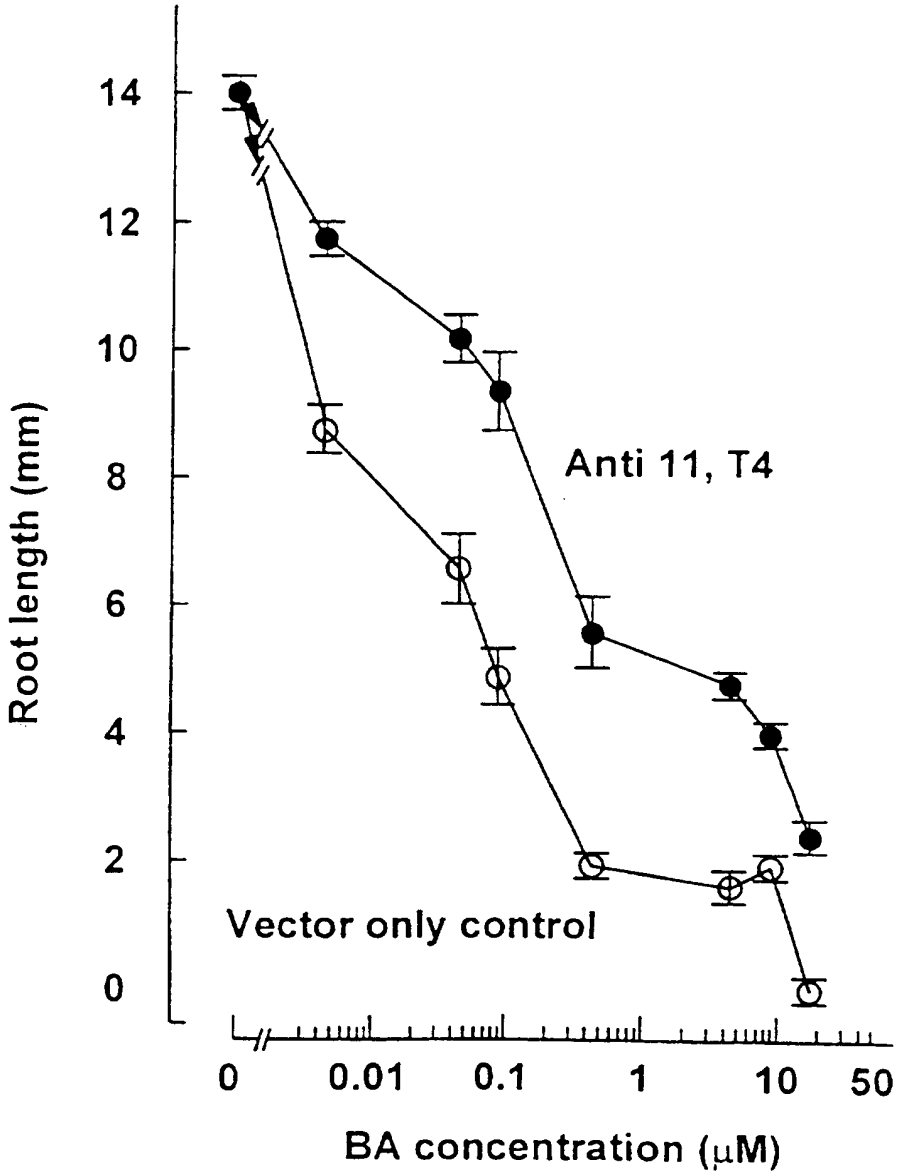


FIG. 12

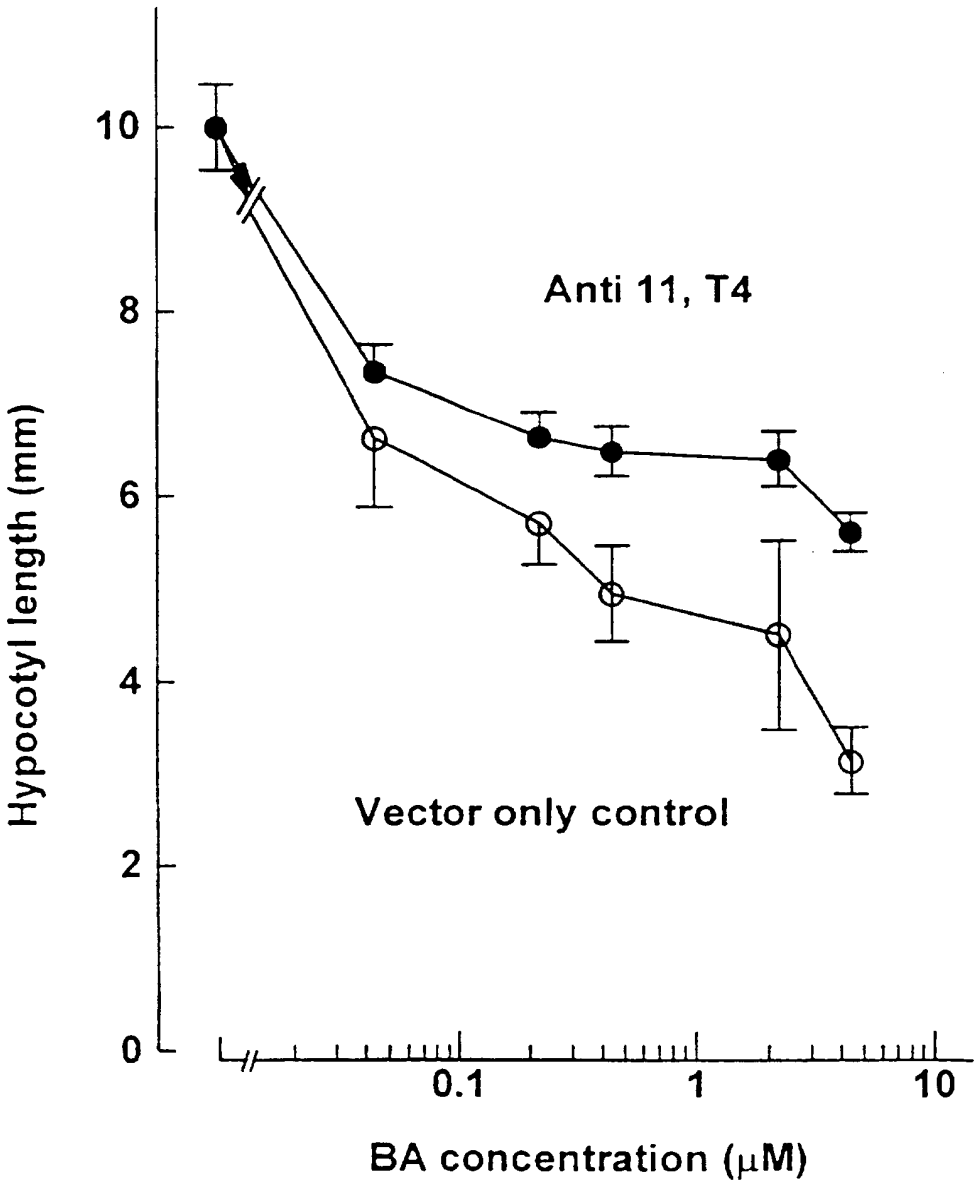


FIG. 13

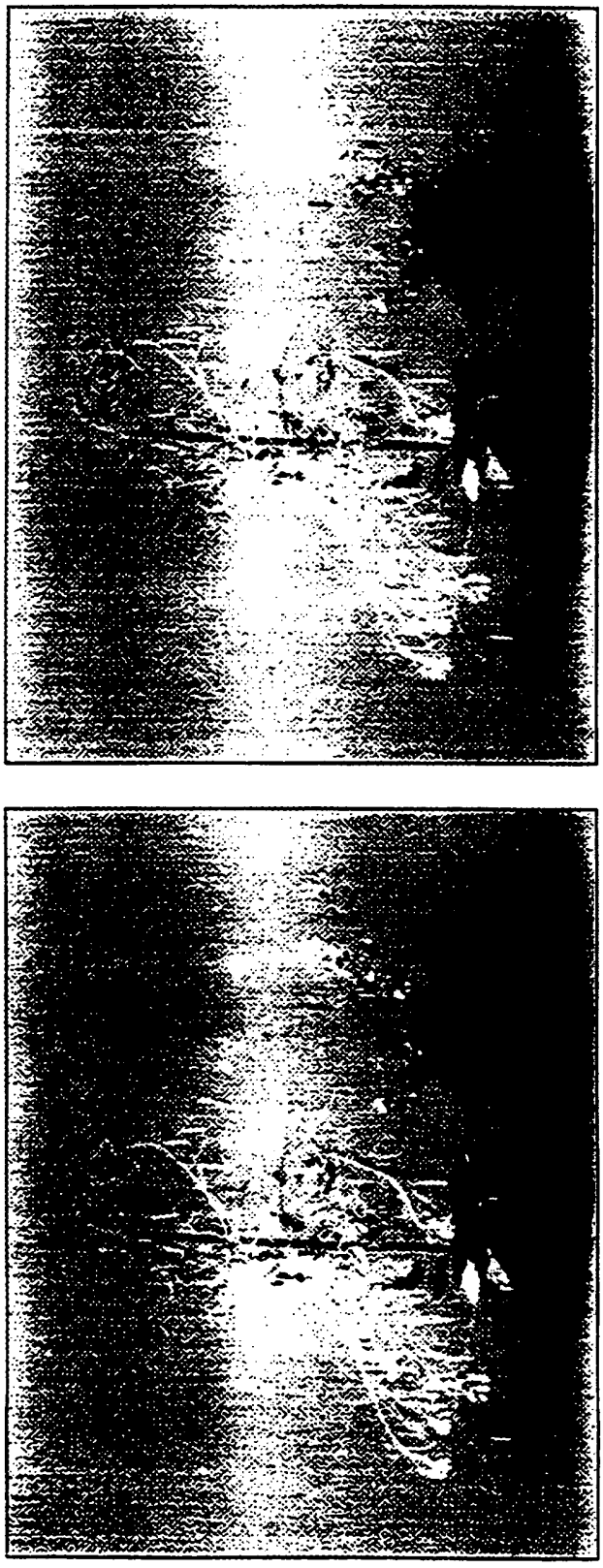


FIG. 14

```
  1  GGTCTTAAAG GAAAGGTACG AGATAATCTT TAGTTTAGCA AAACGATTGG
 51  TTACTACTTA CTACCTGAGT CCTTCTTTTT CTCCAACTTA TGCAGGCTGT
101  TCATTTCTTA ACCTTCTATG CTCCTCTTTG GGGTGCCATT CTCTACAATG
151  GCTTTACATA CTTCCAAGTG ATACGGATGC TAAGAAACGC TAGACGTGTA
201  TGTAATTAAT TGTCTTTTGA AAATATTCAC ATAACCTCAT TTCGACACTT
251  GTCTATCTAC TGACACGGTG GTGTTAAATT TCAGATGGCG GTTGGAATGT
301  CAGACCGAGT GGATCAGTTT GATAATAGAG CAGAGTTAAA GGTTTTTTTC
351  TTCTTCTTCT ATATTGCTAA TACCATTCTG TATATTATGT CTGACTTTGG
401  TTCCTAGATT AAACCTTAAT GCTTGTTTCC TGAAACCACA GGTGTTGAAC
451  CGTTGGGGAT ACTACCCACT CATTCTAATA GGATCATGGG CATTCGGTAC
501  TATCAACCGT A
```

FIG. 15

PCT

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(51) International Patent Classification 6 :
C12N 15/29, 15/11, 15/82, 5/10, 1/21, C12Q 1/68, C07K 14/415, 16/16, A01H 5/00, G01N 33/50

A3

(11) International Publication Number: WO 98/00545

(43) International Publication Date: 8 January 1998 (08.01.98)

(21) International Application Number: PCT/GB97/01766

(22) International Filing Date: 30 June 1997 (30.06.97)

(30) Priority Data:

9613617.1	28 June 1996 (28.06.96)	GB
9706118.8	25 March 1997 (25.03.97)	GB

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

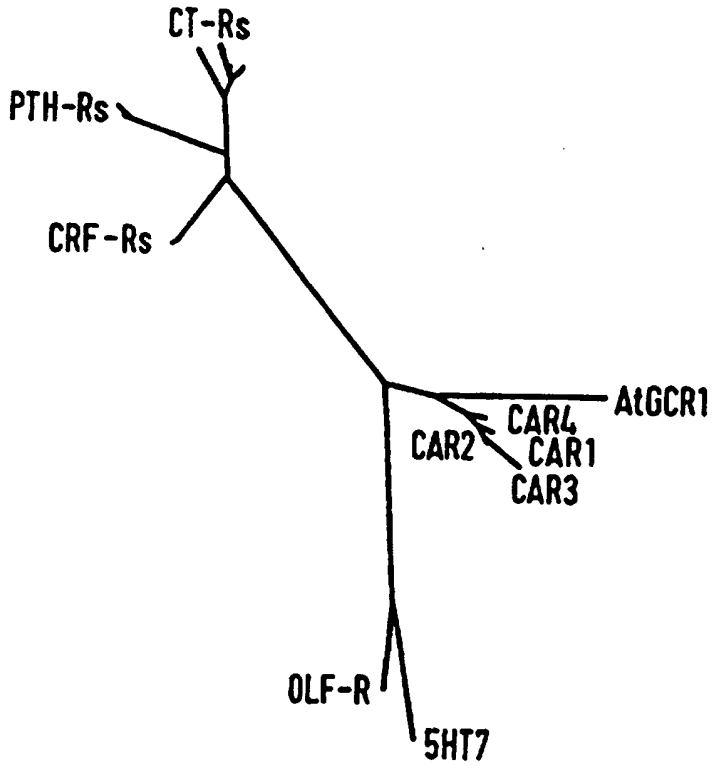
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:
12 March 1998 (12.03.98)

(54) Title: G PROTEIN SIGNAL TRANSDUCTION IN PLANTS

(57) Abstract

A method of altering G protein signal transduction pathways in plants and thereby influencing plant growth, development and responses to endogenous signalling molecules, environmental signals, pests and pathogens is disclosed herein. A G protein-coupled receptor in *Arabidopsis thaliana*, *Brassica napus* and *Brassica oleracea* have been discovered, isolated and cloned. Specifically, the cDNA sequence encoding the full length receptor *Arabidopsis thaliana* has been isolated and purified, as well as the amino acid sequence. The isolated G protein-coupled receptor of the present invention is believed to influence the sensitivity of plants to cytokinins.



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International Application No
GB 97/01766

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/29 C12N15/11 C12N15/82 C12N5/10 C12N1/21
C12Q1/68 C07K14/415 C07K16/16 A01H5/00 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N C12Q A01H G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DESPREZ T. ET AL.: "AC Z33775" EMBL DATABASE, 23 May 1994, HEIDELBERG, XP002050572 see the whole document ---	1-14,40, 41
X	NEWMAN T. ET AL.: "AC T04329" EMBL DATABASE, 30 August 1993, HEIDELBERG, XP002050573 see the whole document ---	1-14,40, 41
X	WISE A. ET AL.: "Isolation of a putative receptor from Zea mays microsomal membranes that interacts with the G-protein, GPalpha1" FEBS LETTERS, vol. 356, no. 2,3, 19 December 1994, pages 233-237, XP002050574 see the whole document ---	17

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[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
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"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 December 1997	15/01/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WEISS C. ET AL.: "Immunolocalization of the G protein alpha subunit encoded by the GPA1 gene in Arabidopsis" THE PLANT CELL, vol. 5, no. 11, November 1993, pages 1513-1528, XP002050575 cited in the application see the whole document	1-47
A	MA H.: "GTP-binding proteins in plants: new members of an old family" PLANT MOLECULAR BIOLOGY, vol. 26, no. 5, December 1994, pages 1611-1636, XP002050576 cited in the application see the whole document	1-47
T	MILLNER P. AND CAUSIER B.: "G-protein coupled receptors in plant cells" JOURNAL OF EXPERIMENTAL BOTANY, vol. 47, no. 301, August 1996, pages 983-992, XP002050577 * see esp. p. 986 ff. *	1-47
T	JOSEFSSON L. AND RASK L.: "Cloning of a putative G-protein-coupled receptor from Arabidopsis thaliana" EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 249, no. 2, October 1997, pages 415-420, XP002050578 see the whole document	1-47